

LEAGUE OF NATIONS

HEALTH ORGANISATION

REPORT

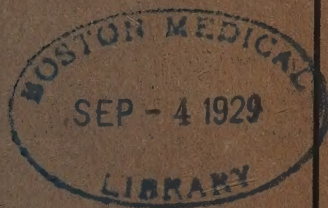
OF THE

SECOND LABORATORY CONFERENCE

ON THE

SERODIAGNOSIS OF SYPHILIS

held at Copenhagen, May 21st to June 4th, 1928



GENEVA, 1928

LEAGUE OF NATIONS

PUBLICATIONS OF THE HEALTH SECTION

BIOLOGICAL AND SEROLOGICAL STANDARDISATION.

- REPORTS ON SEROLOGICAL INVESTIGATIONS presented to the Second International Conference on the Standardisation of Sera and Serological Tests held at the Pasteur Institute in Paris in November 1922 (C. 168. M.98. 1923.III) 8/- \$2.00
- THE STANDARDISATION OF DYSENTERY SERUM by Kiyoshi Shiga, H. Kawamura and H. Tsuychiya. The Kitasato Institute for Infectious Diseases, Tokyo.
- First Report (C.177.M.49.1924.III) 1/6 \$0.40
- Second Report (C.177 (a).M.49 (a). 1924.III) 1/6 \$0.40
- INVESTIGATIONS ON THE SERODIAGNOSIS OF SYPHILIS. Report of the Technical Laboratory Conference, Copenhagen, November 19th to December 3rd, 1923, with two Annexes (French and English texts). (C.5.M.5.1924.III) (C.H.148) 3/6 \$0.80
- REPORT OF THE TECHNICAL CONFERENCE FOR THE CONSIDERATION OF CERTAIN METHODS OF BIOLOGICAL STANDARDISATION. Edinburgh, July 19th to 21st, 1923 (C.4.M.4.1924.III). (C.H.147) 4d. \$0.05
- SECOND INTERNATIONAL CONFERENCE ON THE BIOLOGICAL STANDARDISATION OF CERTAIN REMEDIES convened by the Health Committee of the League of Nations and held at Geneva from August 31st to September 3rd, 1925 (French and English texts). (C.532.M.183.1925.III). (C.H.350) 1/- \$0.25
- THE BIOLOGICAL STANDARDISATION OF INSULIN, including reports on the preparation of the international standard and the definition of the unit (C.H.398.1926.III.7) 1/6 \$0.40

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Geneva, December 1928.

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TABLE OF CONTENTS

	Page
REPORT OF THE SECOND LABORATORY CONFERENCE ON THE SERODIAGNOSIS OF SYPHILIS, HELD IN COPENHAGEN FROM MAY 21ST TO JUNE 4TH, 1928:	
History of the Conference	5
List of Participants	5
Methods compared	7
Explanatory Note on Blood Serum Tests	9
Explanatory Note on the Examination of Cerebro-Spinal Fluids	11
Resolutions	11
Annex 1. — Tables showing the Results of Various Serological Methods:	
Table 1. — Results of the Comparison of Various Serological Methods	30
Table 2. — Positive Reactions in Cases not diagnosed clinically as Syphilis	86
Table 3. — Cerebro-Spinal Fluids	96
Observations on the results obtained:	
Professor D. de Blasí	104
Dr. E. Dehaens	104
Dr. E. Jacobsthal	104
Professor R. Müller	105
Dr. K. Norel	105
Professor R. Otto	105
Professor Sachs	106
Professor A. Vernes	106 and 110
Dr. E. J. Wyler	108
Annex 2. — Description of the Various Methods used	
Professor D. de Blasí	112
Dr. E. Dehaens	115
Dr. L. W. Harrison and Dr. E. J. Wyler	117
Dr. E. Jacobsthal	119
Professor R. Otto and Dr. Blumenthal	120
Dr. D. Pavlovitch	121
Dr. S. Sierakowski	122
Dr. H. Boas	123
Dr. Kahn	123
Professor E. Meinicke	130
Professor R. Müller	133

	Page
Dr. M. Nagayo and Dr. Nobechi	137
Professor H. Sachs and Dr. E. Witebsky.	142
Dr. Th. Madsen and Dr. K. Norel.	147
Vernes Reaction	150
 <i>Appendix.</i> — Method described at the Conference by Dr. A. Scaltritti.	
	151
 <i>Annex 3.</i> — List of the Institutions who sent Blood and Cerebro- spinal Fluid Specimens for the Conference	
	154
 Appendix: COMPARISON OF THE KAHN WITH THE BORDET- WASSERMANN METHOD (No. 1 MEDICAL RESEARCH COUNCIL REPORT [SERIES, No. 14] IN TESTS OF CEREBRO- SPINAL FLUIDS CARRIED OUT BY DR. KAHN AND DR. WYLER IN LONDON AFTER THE CONFERENCE (JULY 10TH-25TH, 1928):	
Table A. — Summary of 317 Tests in which both Workers reported the Specimens as "Clear"	159
Table B. — Summary of 238 Tests in which One or Both Workers reported the Specimens as "Cloudy", as "Con- taminated", or as containing "Some Blood"	160
Table C. — Summary of the 555 Tests shown in Tables A and B.	161
Details of Results included in Table A in which the Kahn and B.-W. Results differed.	162
Details of Results included in Table B in which the Kahn and B.-W. Results differed.	168
Report by Colonel L. W. Harrison.	176
Comments of Dr. R. L. Kahn	180
Remarks concerning Dr. Kahn's Statement by Dr. E. J. Wyler.	183
List of the Names and Addresses of Doctors who sent Specimens of C.S.F. for the Comparison of the Kahn and Wassermann Tests	185



Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis, held in Copenhagen from May 21st to June 4th, 1928.

Since the first Serological Laboratory Conference held at Copenhagen from November 19th to December 3rd, 1923, several of the methods employed for the serodiagnosis of syphilis have improved considerably, and a number of new tests have been elaborated, especially of those depending on directly visible changes in mixtures of syphilitic serum and extract (Flocculation tests).¹ Requests from different Institutes and individuals particularly interested in these problems have therefore been addressed to the Health Organisation with a view to the establishment of a further comparative examination of the methods of serodiagnosis of syphilis. On the proposal of its President, the Health Committee, at its eleventh session in October 1927, approved the convocation of the Second Serological Laboratory Conference on the Serodiagnosis of Syphilis. This Conference was held from May 21st to June 4th, 1928, at the Danish State Serum Institute at Copenhagen. The following attended the Conference, either as experts invited by the Health Organisation or as delegates of Governments or Institutions:

Austria.

Serodiagnost. Untersuchungsanstalt Allgemeines Krankenhaus, Vienna: Professor R. MÜLLER, Dr. R. BRANDT, Mme. S. BERCZELLER.

Denmark.

State Serum Institute, Copenhagen: Dr. Th. MADSEN, Dr. H. BOAS, Dr. J. R. MÖRCH, Dr. K. NORÉL, Mlle. OEJGAARD.

Federated Malay States.

Institute for Medical Research, Kuala-Lumpur: Dr. A. NEAVE KINGSBURY.

¹ See resolutions.

France.

Faculty of Medicine, School of Serology, Paris: Dr. R. DEMANCHE ;
Pasteur Institute, Paris: Dr. E. DEBAINS ;
Prophylactic Institute, Paris: Dr. A. VERNES, M. R. BRICQ,
Mlle. M. KERDAL.

Germany.

Reichsgesundheitsamt, Berlin: Professor H. DOLD ;
Institute "Robert Koch", Berlin: Professor R. OTTO, Dr. G. BLUMENTHAL ;
Serological Institute, Ambrock, Hagen, W.: Dr. E. MEINICKE,
Mme. L. MEINICKE ;
Allgemeines Krankenhaus St. Georg, Hamburg, 5: Dr. E. JACOBSTHAL, Mlle. M. HAUSCHILDT ;
Institut für experimentelle Krebsforschung, Heidelberg:
Professor H. SACHS, Dr. E. WITEBSKY.

Great Britain.

Ministry of Health, London: Dr. L. W. HARRISON, Dr. E. J. WYLER, Mr. E. TWEED.

India.

Medical College, Lucknow: Dr. J. G. MUKERJI.

Italy.

Hygiene Institute of University Royale, Naples: Professor Dante DE BLASI.

Japan.

Government Institute for Infectious Diseases: Professor M. NAGAYO, Dr. K. NOBECHI.

Poland.

State Institute of Hygiene, Warsaw: Professor L. HIRSZFELD,
Dr. S. SIERAKOWSKI, Mlle. H. RABINOWICZ.

Kingdom of the Serbs, Croats and Slovenes.

Serological Service of the Central Hygiene Institute, Belgrade:
Dr. D. PAVLOVITCH, Mme V. KITCHEVATZ.

Sweden.

Pathological Institute, Lund: Professor J. FORSSMAN.

Turkey.

State Institute of Hygiene, Angora: Dr. V. WASSAF.

Union of Socialist Soviet Republics.

State Institute of Experimental Medicine, Epidemiological
Division, Leningrad: Professor D. ZABOLOTNY.

United States of America.

State Hygienic Laboratory, Lansing, and University of
Michigan, Ann Arbor: Professor R. L. KAHN.

Uruguay.

Laboratory of the Prophylactic Institute, Montevideo:
Dr. A. SCALTRITTI.

The following are the names of those invited to the Conference who were unable to accept the invitation:

Argentine.

Public Health Institute, Buenos Ayres: Professor Alfredo
SORDELLI.

Belgium.

Pasteur Institute, Brussels: Dr. RENAUX.

United States of America.

Graduate School of Medicine of the University of
Pennsylvania: Dr. J. A. KOLMER.

The participants were requested to acquaint themselves before the Conference began with the technique of the various methods which were to be compared during their work.

These methods were the following:

1. *The Bordet-Wassermann Reaction (B.-W.R.) represented by Several Modifications:*

- (a) *B.-W.R.* described by Medical Research Council Special Report, Series No. 14, Method No. 1, carried out by Dr. E. J. Wyler, assisted by Mr. E. Tweed.
- (b) *B.-W.R.* described by D. de Blasi, carried out by Professor Dante de Blasi.
- (c) *B.-W.R.* described by Calmette and Massol (modified by Prof. E. Debains, for the examination of non-heated sera), carried out by Prof. Debains.

- (d) *B.-W. R.* synoptic method described by Jacobsthal, carried out by Dr. E. Jacobsthal, assisted by Mlle. M. Hauschildt.
- (e) *B.-W. R.* original method, carried out by Dr. G. Blumenthal.
- (f) *B.-W. R.* original method, carried out by Dr. D. Pavlovitch, assisted by Mme. V. Kitchevatz.
- (g) *B.-W. R.* described by McIntosh and Fildes, carried out by Dr. S. Sierakowsky, assisted by Mlle. H. Rabinowicz.

2. Flocculation tests.

- (a) *Kahn reaction*, carried out by (1) Dr. H. Boas, assisted by Mlle. E. Oejgaard, (2) Professor R. L. Kahn.
- (b) *Meinickes Trübungsreaktion (Meinicke's Turbidity Test) (M.T.R.)*, carried out by Dr. E. Meinicke, assisted by Mme. L. Meinicke.
- (c) *Müllers Ballungsreaktion (Müller's Clotting Test) (M.B.R.)*, carried out by Professor R. Müller, assisted by Mme. S. Berczeller.
- (d) *Murata Reaction*, carried out by Dr. K. Nobechi.
- (e) *Sachs-Georgi (Cito- and Lentochol Reaction)*, carried out by Dr. E. Witebsky.
- (f) *Sigma Reaction modified*, carried out by Dr. K. Norél.
- (g) *Vernes Syphilimetric Reaction*, carried out by M. R. Bricq, assisted by Mlle. M. Kerdal.

A detailed description of the techniques of the various methods in question is given in Annex 2 of this report.

The samples of blood and spinal fluid were placed at the disposal of the Conference by a number of Danish hospitals and dispensaries, as well as by clinics in Berlin, Hamburg, London, Vienna and Paris (see Annex 3). As the result of the Conference depended entirely upon the sufficiency and variety of the material placed at its disposal, its cordial thanks are due to the chief physicians of the different clinics who so

generously offered their work and these samples for the use of the participants.

The origin of the samples tested is indicated in the Tables attached to this report (Annex 1) and where no indication is given the sample was collected at Copenhagen. The specimens received from abroad were despatched to the Copenhagen Institute by air mail, and were kept in ice until their distribution. Owing to the time taken in transit and that required for their preparation prior to distribution, the sera from abroad were from three to nine days old, whilst the Danish sera were quite fresh. Thus the material before the Conference consisted, as is usually the case in the routine work of large diagnostic centres, of both old and fresh sera.

The clinical diagnosis of the sera submitted for the examination of the Conference were not published until the participants had submitted their reports on the results obtained, the sera and spinal fluids being distributed without any indication as to their origin or the diagnosis. In the numerous cases in which the clinical diagnosis was uncertain, and where discrepancies occurred between the serological findings and the clinical diagnoses, the case was thoroughly examined by Dr. A. K. H. CORNING. In cases showing one or more positive sero-reactions, if no history of syphilis was available, a new sample of blood was withdrawn where possible and the serum retested. The Conference is greatly indebted to Dr. Corning for his valuable aid.

A. BLOOD SERUM TESTS.

During the Conference, 944 sera ¹ were tested by various methods. Of these, 502 were derived from known cases of syphilis in all stages of the disease both treated and untreated, seven were derived from cases with no definite history of syphilis, but where there was nevertheless some evidence of syphilis. 435 sera were derived from patients suffering from various other diseases (tuberculosis, cancer, scarlet fever, gonorrhœa; pregnancy, etc.) but with no clinical signs of syphilis. It must be remembered, however, that, as experience

¹ In addition to these, thirteen blood sera were examined by a limited number of participants after the actual close of the Conference (Sera Nos. 945-957).

shows, a certain number of the latter cases may nevertheless be syphilitic.

The detailed results of the tests carried out by the different methods are presented in *Table 1* of Annex 1, prepared on the basis of the daily reports furnished by each worker immediately after having read the results. In *Table 2* of Annex 1 are collected the detailed results of the tests which, with one or more of the methods, gave a positive reaction in cases without clinical signs of syphilis.

It should be noted that, in judging the results obtained, it was not considered the purpose of the Conference to recommend any special method. It must be remembered that the choice of a method should depend on various considerations especially with regard to sensitiveness and specificity, and also as to local conditions, time required, cost. It is hoped, however, that the tables referred to below, which contain indications with regard to sensitiveness and specificity, will facilitate the analysis of the results.

In summarising the results of the different tests, some difficulty arose from the seven sera mentioned above with uncertain diagnosis. These cases, although without any definite history of syphilis, showed strong evidence of being nevertheless syphilitic; but, as the clinical diagnosis of syphilis could not be established with certainty, it was thought best to count them neither as "syphilitic" nor as "non-syphilitic", but to classify them in a special group as "doubtful". The detailed results of the tests in these cases (performed both during and after the close of the Conference) are given in *Table (i)* with a brief clinical report. A summary of the results of the different methods in respect of the *total number* of cases examined (944) is presented in *Table (a)*. It will be noted that the cases are divided into three groups: syphilitic, doubtful and non-syphilitic.

The results of the different methods in question can only be compared in cases when the tests on sera were examined by *all* the workers. Unfortunately, a considerable number of sera were not tested by all of the participants and, in order to obtain a *direct* comparison, it would be necessary to reject a considerable part of the material available. The following procedure — that of *indirectly* comparing the results — was therefore adopted.

The results obtained by the majority of the participants (ten) who tested all or practically all the 944 sera, are compared directly, omitting the small number of sera (six cases of syphilis, six non-syphilitic) which were not tested by one or another of these workers. The tests of the other workers (five), who did not examine more than a limited number of sera, are then each compared individually with the corresponding sera tested by the principal group of workers.

The details of the tests in the twelve sera omitted from the comparison are given in *Table (k)*. It is obvious that this omission does not change the relative position of the methods with regard to their apparent value.

The results of the group of methods which are directly comparable is given in *Table (b)*.

The results of those methods which are compared separately in their relationship to the main group are given in *Tables (c)–(h)*.

B. CEREBRO-SPINAL FLUIDS.¹

The detailed results of the examination of cerebro-spinal fluids is presented in *Table 3* of Annex 1. As the number of samples received was relatively small (122) and owing to the fact that in several cases the quantity of fluid available was insufficient, a definite conclusion can hardly be drawn from the results obtained by the Conference, especially as, owing to the time taken in transit from abroad, a considerable proportion of the material was not suitable for examination. Tables have been drawn up on the basis of the daily reports of the investigators, but no summary of the results was made.

The following resolutions were adopted:

RESOLUTIONS.

I.

The Conference,

Having considered the results of the blood-serum² tests for syphilis according to the methods under review:

¹ See also Appendix.

² The material available at this Conference has not been sufficient to permit of a conclusive comparison between the Bordet-Wassermann and the flocculation tests in the examination of cerebro-spinal fluid.

Notes that in the case of those which depend on directly visible changes in mixtures of extract and serum, hereinafter referred to as flocculation¹ tests certain new tests have been elaborated and certain others have improved considerably since the last Conference in 1923;

And is of opinion that the best of them may be regarded as equal in value to the best of those which depend on fixation of complement (Bordet-Wassermann).

It desires nevertheless to emphasise the fact that, no less than the complement-fixation tests, these flocculation methods are, despite their apparent simplicity, extremely sensitive to the slightest differences in experimental conditions and subject to so many sources of error, in connection both with the execution of the test and the reading and interpretation of the results, that they must be placed only in the hands of specially trained serologists.

II.

The Conference,

Being of opinion that some serological tests may have the advantage of greater sensitiveness without being absolutely specific, and *vice versa*, and that concordance of reaction to two or more tests has greater diagnostic value than has a single reaction:

Recommends that, in order to secure the most reliable information to the clinician, at least two different sero-diagnostic methods should be used².

¹ The term "flocculation" is employed here for convenience without prejudice to opinions as to whether any given reaction is one of flocculation, increase of turbidity, precipitation, or clotting. It is applied here to the following tests: Kahn, Meinicke (M.T.R. and M.M.R.), Müller (M.B.R.), Murata, Sachs-Georgi, Sigma and Vernes.

² The following, namely: Dr. de Blasi, Dr. Debains, Dr. Demanche, Dr. Dold, Dr. Harrison, Dr. Hirzsfeld, Dr. Jacobsthal, Dr. Kingsbury, Dr. Meinicke, Dr. Muckerji, Dr. Müller, Dr. Nagayo, Dr. Otto, Dr. Pavlovitch, Dr. Sachs, Dr. Scaltritti and Dr. Zabolotny, considering:

(a) That, as theoretical considerations give reason to expect, some sera react to the Bordet-Wassermann but not to the flocculation tests and *vice versa*, and that the Bordet-Wassermann and the flocculation tests supplement each other;

(b) That strong confirmation of a weak or \pm flocculation test is afforded by a positive Bordet-Wassermann test, and *vice versa*,

Would for the present prefer that one of the methods should be a Bordet-Wassermann test.

III.

The Conference,

Having in view the necessity for constantly readjusting serodiagnostic methods in order to obtain the highest degree of specificity :

Recommends that the serologist should check the accuracy of his tests by regular and very frequent reference to clinical data, in consultation with the clinician, whose assistance in supplying adequate information as to the history of syphilis and the clinical particulars of the case is of great value for the interpretation of the results.

IV.

The Conference,

Having in view the fact that serological tests for syphilis are primarily for the purpose of assisting clinicians in diagnosis, in observing progress under treatment and in tests for cure;

And having in view also the fact that patients frequently pass from the care of one clinician to that of another, the serum of one patient being tested from time to time in different laboratories:

Is of opinion that a uniform method of notation of serological results bearing approximately the same clinical interpretations would be of great value to clinicians, and proposes the following general rules:

(1) That a negative reaction should be reported as “—” or “negative”;

(2) That a reaction which is just positive to a degree which in the hands of the serologist has been afforded practically only by sera from cases of syphilis (and of a few well-defined pathological conditions) should be reported as “+” or “positive”.

It is recommended, in this connection, that serologists should so adjust their tests that practically only sera from cases of syphilis afford reactions which they report as “+” or “positive” ;

(3) That a reaction which is neither negative nor positive as defined in (2) should be reported as “ \pm ”.

In making these recommendations, the Conference would remark that there is nothing in them which would prevent the serologist adding to his report any amplifying or explanatory note which may be considered desirable (*e.g.* signs expressing the strength of the reaction).

V.

The Conference wishes to reiterate with particular emphasis:

(1) That, in spite of the increased sensitiveness which the various serodiagnostic methods have shown at the present Conference, serological results may, notwithstanding the presence of a syphilitic infection, be negative in certain cases;

(2) That a positive reaction in the absence of a clear history or of signs of syphilis should, if only to exclude all possibility of error, never be accepted until a test of at least one more specimen has afforded the same result;

(3) That, except in the case of a few well-defined pathological conditions, syphilis is indicated with a degree of probability which closely approaches certainty, when several tests performed according to different methods give a positive result.

The Conference would suggest that the gist of the remarks in sub-sections (1) to (3) might be printed on the backs of the reports on serum tests for syphilis which are rendered by serologists to clinicians.

VI.

The Conference,

Having in view the special importance of serodiagnosis for the diagnosis, treatment and prevention of syphilis:

Desires to record its view that considerable misunderstanding would be avoided and reports on tests of sera would be greatly enhanced in value if clinicians would study closely the diagnostic and therapeutic implications of such reports.

VII.

The Conference,

Considering that the work in common and the discussions among participants have contributed greatly to a fuller knowledge and better understanding of the subject;

Bearing in mind, however, that the methods for the serodiagnosis of syphilis are constantly improving and that they are of capital importance for public health, and in order to stamp out a social scourge:

Considers it extremely desirable that the Health Organisation of the League of Nations should keep this question on the programme and take steps to secure further comparisons of this kind in the future.

VIII.

The Conference,

Being of opinion that the special value of its work lies in the fact that the authors have themselves been able to compare, on the same test material, the results of their own methods with those of others, and contemplating the possibility of ultimately securing uniformity in the serodiagnosis of syphilis;

Holds it to be desirable that the Danish State Serum Institute, acting as the central laboratory of the Health Organisation of the League of Nations, should, in continuation of the work of the Conference,

Undertake the distribution of a series of serum samples for comparative tests in the different laboratories;

Itself test, at request, any other serum samples which it may receive, or distribute them for purpose of comparative re-testing;

And undertake to arrange in the same way an exchange of extracts, thus initiating a co-ordination of work which might be further developed in due course.

Tableau a).

SÉRUM SANGUIN.

Résumé des résultats obtenus par l'examen du total des cas
(944 cas)¹.

Réaction Reaction	Effectuée par Carried out by	Cas syphilitiques (502 cas) Syphilitic cases (502 cases)					
		Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & ++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done
B.-W. R.	De Blasi	461	130	72	259	0	41
»	Debains	315	167	9	135	4	187
»	Harrison-Wyler . .	502	210	78	214	0	0
»	Jacobsthal	502	265	65	172	0	0
»	Otto-Blumenthal . .	502	260	65	176	0	1
»	Pavlovitch	501	220	56	225	1	0
»	Sierakowski	502	195	57	250	0	0
Kahn R.	Boas	502	294	27	181	0	0
»	Kahn	499	305	33	161	0	3
M.T.R.	Meinicke	502	246	38	218	0	0
M.B.R.	Müller	499	317	45	137	0	3
Murata R.	Nagayo-Nobechi . .	497	255	67	155	20	5
S.G.R. lento.	Sachs-Witebsky . .	497	208	23	253	13	5
» cito.	Sachs-Witebsky . .	496	254	21	209	13	6
Sigma R.	Norél	502	257	76	169	0	0
Vernes R.	Vernes-Bricq	453	174	62	198	19	49

¹ Y compris les sérums réexaminés.

² Cas sans historique défini de syphilis, mais qui sont néanmoins suspects; voir tableau i) (détails sérologiques) et rapports cliniques.

Table (a).

BLOOD SERUM

Summary of results obtained with the total amount of cases examined (944 cases)¹.

Cas douteux (7 cas) Doubtful cases ² (7 cases)						Cas non syphilitiques (435 cas) Non-syphilitic cases (435 cases)					
Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & +++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done	Nombre d'épreuves effectuées Number of tests carried out	Nombre de Number of + & +++	Nombre de Number of ±	Nombre de Number of —	Nombre d'épreuves à refaire Number of tests to be repeated	Nombre d'épreuves non effectuées Number of tests not done
5	0	2	3	0	2	397	13	34	350	0	38
4	2	0	2	0	3	249	26	4	213	6	186
7	3	2	2	0	0	435	0	12	423	0	—
7	3	2	2	0	0	435	29	36	369	1	—
7	3	3	1	0	0	435	24	40	371	0	—
7	3	1	3	0	0	434	6	25	403	0	1
7	3	2	2	0	0	435	0	13	422	0	—
7	6	0	1	0	0	435	3	6	426	0	—
7	6	0	1	0	0	434	0	5	429	0	1
7	7	0	0	0	0	435	9	13	413	0	—
7	6	0	1	0	0	432	1	10	421	0	3
7	4	1	2	0	0	432	2	36	372	22	3
7	4	0	2	1	0	431	0	1	430	1	4
7	4	1	1	1	0	427	0	1	425	1	8
7	4	2	1	0	0	434	6	35	393	0	1
5	2	1	2	0	2	369	2	36	316	15	66

¹ Including re-tested sera.

² Cases with no definite history of syphilis but with some evidence of being syphilitic; see table (i) (serological details) and clinical reports.

Tableau b).

SÉRUMS SANGUINS.

Comparaison des résultats obtenus
au moyen des différentes méthodes.

Table (b).

BLOOD SERA.

Comparison of results obtained by
means of the different methods.

Méthode Method	Exécutée par Carried out by	Nombre de + et de ++ parmi les cas diagnostiqués cliniquement comme: Number of + and ++ in cases with the clinical diagnosis:		
		Syphilis (496 cas) (496 cases)	Douteux Doubtful (7 cas) (7 cases)	Sans syphilis Non-syphilitic (429 cas) (429 cases)
Bordet-Wassermann	De Blasi	Voir tableau c) See table (c)		
Bordet-Wassermann (Calmette-Massol)	Debains	Voir tableau d) See table (d)		
Bordet-Wassermann (Méthode 1) (Method 1) "Medical Research Council Report Series No. 14"	Harrison-Wyler . .	208	3	0
Bordet-Wassermann (Méthode synoptique) (synoptic method)	Jacobsthal	261	3	28
Bordet-Wassermann (originale) (original)	Otto-Blumenthal .	256	3	24
Bordet-Wassermann (originale) (original)	Pavlovitch	218	3	6
Bordet-Wassermann (McIntosh-Fildes)	Sierakowski	192	3	0
Kahn	Boas	289	6	3
Kahn	Kahn	303	6	0
Meinicke (Réaction d'opaci- fication) (Turbidity test)	Meinicke	241	7	9
Müller (Réaction de conglomé- ration) (Clotting test)	Müller	314	6	1
Murata	Nagayo-Nobechi . .	Voir tableau e) See table (e)		
Sachs-Georgi (Lentochol)	Sachs-Witebsky . .	Voir tableau f) See table (f)		
Sachs-Georgi (Citochol)	Sachs-Witebsky . .	Voir tableau g) See table (g)		
Sigma	Norél	253	4	6
Vernes	Vernes	Voir tableau h) See table (h)		

Tableau c).

Le professeur DE BLASI (B.-W.R.)
a examiné :

455 sérums provenant de cas syphil.
5 » » » douteux.
391 » » » non syphil.

Ce tableau montre les résultats obtenus dans ces cas par le professeur de Blasi, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

Table (c).

Professor DE BLASI (B.-W.R.)
examined :

455 sera from syphilitic cases.
5 „ „ „ doubtful „ „
391 „ „ „ non-syphilitic cases.

This table shows the results obtained in these cases by Professor de Blasi, compared with the corresponding results obtained by the other methods.

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	De Blasi	129	0	13
B.-W.R.	Harrison-Wyler . . .	184	1	0
B.-W.R.	Jacobsthal	241	2	28
B.-W.R.	Otto-Blumenthal . . .	233	1	23
B.-W.R.	Pavlovitch	201	2	6
B.-W.R.	Sierakowski	173	2	0
Kahn R.	Boas	263	4	3
Kahn R.	Kahn	279	4	0
M.T.R.	Meinicke	219	5	9
M.B.R.	Müller	289	4	1
Sigma R.	Noréi	234	4	6

Tableau d).

Le Dr DEBAINS (B.-W.R.)
a examiné:

311 sérums provenant de cas syphil.
4 » » » douteux.
244 » » » non syphil.

Ce tableau montre les résultats obtenus dans ces cas par le Dr Debains, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

Table (d).

Dr. DEBAINS (B.-W.R.)
examined:

311 sera from syphilitic cases.
4 „ „ „ doubtful „
244 „ „ „ non-syphilitic cases.

This table shows the results obtained in these cases by Dr. Debains, compared with the corresponding results obtained by the other methods.

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	Debains	164	2	26
B.-W.R.	Harrison-Wyler . . .	126	1	0
B.-W.R.	Jacobsthal	172	1	13
B.-W.R.	Otto-Blumenthal . . .	162	1	12
B.-W.R.	Pavlovitch	148	2	6
B.-W.R.	Sierakowski	118	2	0
Kahn R.	Boas	184	3	1
Kahn R.	Kahn	202	3	0
M.T.R.	Meinicke	156	4	7
M.B.R.	Müller	207	3	1
Sigma R.	Norél	171	3	5

Tableau (c).

Les Drs NAGAYO et NOBECHI (Murata R.) ont examiné:

461 sérum provenant de cas syph.
 7 " " " " douteux.
 426 " " " " non syph.

Ce tableau montre les résultats obtenus dans ces cas par les Drs Nagayo et Nobechi, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

Table (c).

Drs. NAGAYO and NOBECHI (Murata R.) examined:

461 sera from syphilitic cases.
 7 " " " " doubtful " "
 426 " " " " non-syphilitic cases.

This table shows the results obtained in these cases by Drs. Nagayo and Nobechi, compared with the corresponding results obtained by the other methods.

Méthode Méthode	Examinés par Examined by	Cas syphilitiques Syphilitic cases - & - -	Cas douteux Doubtful cases - & - -	Cas non syphilitiques Non-syph. non-cases - & - -
B. W. R.	Harrison-Wyler	265	3	0
B. W. R.	Leubnitzel	259	3	26
B. W. R.	Geo-Bismuthel	254	3	24
B. W. R.	Parkvitch	216	3	6
B. W. R.	Nakazonoki	196	3	9
Kahn R.	Ross	266	6	3
Kahn R.	Kahn	298	6	9
M. T. R.	Melnike	241	7	6
M. R. R.	Munk	319	6	1
Murata R.	Nagayo-Nobechi	251	7	2
Nagayo R.	Nobé	251	4	6

Tableau f).

Les Drs SACHS et WITEBSKY (S.G.R.
Lentochol) ont examiné:

491 sérums provenant de cas syphil.
7 » » » douteux.
425 » » » non syphil.

Ce tableau montre les résultats obtenus dans ces cas par les Drs Sachs et Witebsky, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

Table (f).

Drs. SACHS and WITEBSKY (S.G.R.
Lentochol) examined:

491 sera from syphilitic cases.
7 „ „ „ doubtful „
425 „ „ „ non-syphilitic cases.

This table shows the results obtained in these cases by Drs. Sachs and Witebsky, compared with the corresponding results obtained by the other methods.

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases + & ++
B.-W.R.	Harrison-Wyler . . .	206	3	0
B.-W.R.	Jacobsthal	258	3	27
B.-W.R.	Otto-Blumenthal . . .	253	3	24
B.-W.R.	Pavlovitch	216	3	5
B.-W.R.	Sierakowski	190	3	0
Kahn R.	Boas	285	6	3
Kahn R.	Kahn	299	6	0
M.T.R.	Meinicke	239	7	9
M.B.R.	Müller	311	6	1
S.G.R. Lento.	Sachs-Witebsky . . .	206	4	0
Sigma R.	Norél	250	4	6

Tableau g).

Les Drs SACHS et WITEBSKY (S.G.R. Citochol) ont examiné:

490 sérums provenant de cas syphil.
 7 » » » douteux.
 421 » » » non syphil.

Ce tableau montre les résultats obtenus dans ces cas par les Drs Sachs et Witebsky, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

Table (g).

Drs. SACHS and WITEBSKY (S.G.R. Citochol) examined:

490 sera from syphilitic cases.
 7 „ „ „ doubtful „
 421 „ „ „ non-syphilitic cases.

This table shows the results obtained in these cases by Drs. Sachs and Witebsky, compared with the corresponding results obtained by the other methods.

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphilitic cases + & ++
B.-W.R.	Harrison-Wyler . . .	205	3	0
B.-W.R.	Jacobsthal	257	3	27
B.-W.R.	Otto-Blumenthal . . .	252	3	23
B.-W.R.	Pavlovitch	215	3	5
B.-W.R.	Sierakowski	189	3	0
Kahn R.	Boas	284	6	3
Kahn R.	Kahn	298	6	0
M.T.R.	Meinicke	238	7	8
M.B.R.	Müller	310	6	1
S.G.R. Cito.	Sachs-Witebsky . . .	250	7	0
Sigma R.	Noré	249	4	6

Tableau h).

Le professeur VERNES et M. BRICQ
(méthode syphilimétrique)
ont examiné :

448 sérums provenant de cas syphil.
5 » » » douteux.
365 » » » non syphil.

Table (h).

Professor VERNES and M. BRICQ
(Syphilimetric method)
examined :

448 sera from syphilitic cases.
5 „ „ „ doubtful „
365 „ „ „ non-syphilitic cases.

Ce tableau montre les résultats obtenus dans ces cas par le professeur Vernes et M. Bricq, comparés aux résultats correspondants obtenus à l'aide des autres méthodes.

This table shows the results obtained in these cases by Professor Vernes and M. Bricq, compared with the corresponding results obtained by the other methods.

Réaction Reaction	Examiné par Examined by	Cas syphilitiques Syphilitic cases + & ++	Cas douteux Doubtful cases + & ++	Cas non syphilitiques Non-syphi- litic cases. + & ++
B.-W.R.	Harrison-Wyler	190	3	0
B.-W.R.	Jacobsthal	234	2	22
B.-W.R.	Otto-Blumenthal	231	3	17
B.-W.R.	Pavlovitch	198	2	5
B.-W.R.	Sierakowski	172	2	0
Kahn R.	Boas	264	4	3
Kahn R.	Kahn	277	4	0
M.T.R.	Meinicke	225	5	5
M.B.R.	Müller	287	4	1
Sigma R.	Norél	234	4	6
Vernes R.	Vernes-Bricq	171	2	2

Tableau i)

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Table (i)

Tableau i)

RÉACTIONS SÉROLOGIQUES DANS LES CAS CLASSÉS COMME DOUTEUX:

Date	Sérum No.	B.-W. R.						
		De Blasi	Debains	Harrison- Wyller	Jacobsthal	Otto- Blumenthal	Pavlovitch	Sierakowsky
21.V 14.VI 14.VII	60 ¹ 60 60	0	0	++	+	++	++	+
21.V 1.VI 14.VI	80 ¹ 80 (838) 80 (838)	0 —	0 0	+ ±	± ++	++ ±	— ±	± ±
25.V	418	±	++	++	++	++	++	++
29.V 2.VI	490 ¹ 490(953)	— 0	— 0	— ±	— 0	± +	— 0	— 0
30.V 14.VII	657 ¹ 657	±	—	—	—	—	—	—
31.V 2.VI 15.VI	709 ¹ 709(952) 709(952)	—	++	± ±	±	±	++	+

¹ Les Sérums ont été examinés plusieurs fois (voir dates). — These sera have been tested several times (see dates).

² M. T. R. effectués par le D^r Boas. — M. T. R. tested by Dr. Boas.

HISTORIQUE CLINIQUE DES CAS CLASSÉS COMME DOUTEUX.

- N^o 60. Femme M., née en 1910. Diagnostic: tuberculose pulmonaire, urétrite gonococcique. Tuberculose floride bilatérale. Température 38,5° C. Crachats + T.B.C. Sécrétion urétrale + pour gonocoque. Pas de signes cliniques de syphilis. Décédée le 14.IX.1928. Autopsie: pas de lésions syphilitiques.
- N^o 80. Homme I., né en 1887. Diagnostic tuberculose pulmonaire avec bronchiectasie. En traitement par la sanocrysine. Temp. 38,5° C. Nic la syphilis; pas de signes cliniques de cette maladie.
- N^o 418. Homme P., né en 1903. Diagnostic: chancre mou. Pas d'anamnèse syphilitique. Recherche des spirochètes dans le chancre négative. Malade en observation à la clinique, en attendant des manifestations de syphilis. Réaction Bordet-Wassermann + à la clinique (Berlin).
- N^o 490. Homme O. E., né en 1903. Diagnostic: tuberculose pulmonaire. En 1921: chancre pénien trouvé à l'hôpital Frederiksberg. Frottis répétés avec résultats négatifs. Réaction Bordet-Wassermann faiblement positive. En 1922: réaction B.-W. négative; pas de cicatrices péniennes du chancre; pas d'exanthème.
- N^o 657. Femme K., née en 1861. Diagnostic clinique: « cancer rectal ? ». Diagnostic autopsique: cholélithiasie, cholécystite chronique et aigue. Extrait de l'observation: A la clinique R.B.-W. négative pour le sang; liquide céphalo-rachidien non examiné. Réaction à la luétine (+); pupilles normales. Réflexes achilléens et patellaires normaux; Babinski positif à droite, négatif à gauche. Réaction d'Oppenheim positive à droite, négative à gauche. Pas d'ataxie. A la peau du tronc: lésions eczématiformes, serpiginieuses, avec rebord croûteux. Ces lésions, d'apparence syphilitique au début, cédèrent à un traitement local cutané, mais des nodules indurés restèrent perceptibles sous la peau.
- N^o 709. Femme L., née en 1895. Diagnostic: pleurésie exsudative gauche. Tuberculose laryngée? Infiltration marquée des deux cordes vocales, sans bacilles tuberculeux. Pas de syphilis dans l'anamnèse, ni de signes cliniques de cette maladie. (La lésion laryngée est-elle syphilitique?). Réaction d'Herxheimer après injection réactivante de 0 gr. 15 de salvarsan.

Table (i)

SEROLOGICAL REACTIONS IN CASES REFERRED TO AS DOUBTFUL

Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.	B. W. R. Statens Serum Institut
Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs-Witebsky	Noré	Vernes-Bricq	Boas
++ ++ ++	++	++	++	++	++	+ + +	+	++ ++ ++
++ ++ ++	++ ++	++ ++ ++ ²	++ ++	++ —	++ E.Fl.	± + +	± 0	+ + ++
++	++	++	++	++	++	+	+	++
— 0	— —	++ +	— 0	— 0	— ± 0	— —	— 0	
++ ++	++	+ ++ ²	+	±	—	± ++	—	0 ++
++ ++ ++	++ ±	++ ++ ++ ²	++	++	+	+ + +	0	++ ++ ++

CLINICAL HISTORY OF CASES REFERRED TO AS "DOUBTFUL".

- No. 60. Female M., born in 1910. — Diagnosis: pulmonary tuberculosis, gonococcal urethritis. Active bilateral tuberculosis. — Temperature 38.5° C. Sputum + tuberculosis. — Urethral secretion + for gonococci. No signs of clinical syphilis. Died on 14.IX.1928. — Post-mortem: no syphilitic lesions.
- No. 80. Male I., born in 1887. — Diagnosis: pulmonary tuberculosis with bronchiectasis. Under treatment with Sanocrysin. — Temperature 38.5° C. Denies syphilis; no clinical signs of this illness.
- No. 418. Male P., born in 1903. — Diagnosis: soft chancre. No patient's history of syphilis. Examination of the chancre for spirochetes: negative. Patient under observation in a clinic for manifestation of syphilis. Reaction Bordet-Wassermann done at the clinic (Berlin): positive.
- No. 490. Male O.E., born in 1903. — Diagnosis: pulmonary tuberculosis. In 1921: penile chancre found at the hospital Frederiksberg. Repeated slides with negative results. Bordet-Wassermann reaction feebly positive. In 1922: B.-W. reaction: negative; no penile cicatrices of the chancre; no rash.
- No. 657. Female K., born in 1861. — Clinical Diagnosis "rectal cancer" ? P. M. diagnosis: cholelithiasis, cholecystitis chronic and acute. Extract from the note: At the clinic: B.-W. reaction for blood: negative. Cerebro-spinal fluid not examined. Reaction to luetine: positive; pupils normal. Patella and ankle reflexes normal. Babinski sign, positive on the right side, negative on the left. Oppenheim reaction: positive on the right side, negative on the left. No ataxia. On the skin of the trunk: serpiginous eczematous lesions surrounded with crusts. These lesions of a syphilitic appearance at the beginning, yielded to local skin treatment, but hard nodules remained perceptible under the skin.
- No. 709. Female L., born in 1895. — Diagnosis: Pleurisy with exsudation, on the left side. Tuberculous laryngitis ? Marked infiltration of the two vocal cords. No tubercle bacilli. No history of syphilis and no clinical signs of this illness. (Was the laryngeal lesion syphilitic ?) Reaction of Herxheimer after an activating injection of 0.15 gr. of salvarsan.

Annex I.

TABLES SHOWING THE RESULTS OF THE COMPARISON OF VARIOUS SEROLOGICAL METHODS.

The hieroglyphics used in the tables:

\ddagger = strong positive = > 3.9 Σ units in 22 hours = > 10
Vernes units.

$+$ = positive = 1.5—3.9 Σ units in 22 hours = 6-10
Vernes units.

\pm = neither negative nor positive = 1.0—1.4 Σ units in
22 hours = 1-5 Vernes units.

$-$ = negative = < 1.0 Σ units in 22 hours.

$?$ = doubtful.

0 = test not done.

C = Chylosis, H = Haemolysis, E.Fl. = Spontaneous
flocculation.

The \bullet^t in the tables under "Diagnosis" indicates that
syphilitic cases had been treated.

\square = positive reaction in cases not diagnosed clinically
as "Syphilis".

N. V. D. = no venereal disease.

N.H. Syph. = no history of syphilis.

θ = Naught.

A ref. = to be repeated.

Method used by Dr. E. Debains

Record of intensity of reactions.

(A = minimal dose of complement).

Dose of complement determined by the serum examined:

A₁ = Very weak

A₂ = Weak

A₃ = Medium

A₄ = Rather strong

A₅ = Strong

$>A_5$ = Very strong.

Method used by Sachs-Witebsky.

If two hieroglyphics are indicated, the first refers to the
Lentochol, the second to the Citocholreaction.

Table (1)

RESULTS OF THE COMPARISON OF VARIOUS SEROLOGICAL METHODS.

Diagnosis										Observations	No.
Syphilis						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae	
								•		Gon. Condylom. Lupus vulg. Rheumatismus Condylom. Lues cerebrospinalis.	= n° 822
		• t	• t					•		Aneurism. aortae Cancer recti Cancer ventr. Puerperium Bronchit. chr.	= n° 869
		• t							•	Neuralgia	
			• t				•				
			• t				•				
			• t				•				
			• t				•				
			• t				• ?				
					• t		•			Lues cerebrospinalis.	
		• t					•			Lues cerebrospinalis.	
		• t								Lues cerebrospinalis. Neuritis Apoplexia cerebr.	
• t											
		• t									
			• t							Gon.	
			• t								
			• t							Scarlatina	
								•			
										Balanitis.	
			• t								
								•		Cancer uteri Pleuro-pneumonia Ischias	

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R	
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinike	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq	
21/5	36	0	0	+	+	+	+	+	+	+	+	+	+	+	1.6	+	48
	37	0	0	-	-	-	-	-	-	-	-	-	-	-	± 1.3	-	θ
	38	0	0	-	±	-	-	-	-	-	-	-	-	-	-	-	θ
	39	0	0	-	-	-	-	-	-	-	-	-	-	-	± 1.1	-	θ
	40	0	0	-	-	-	-	-	-	-	-	-	-	0	-	-	θ
*	41	0	0	+	+	+	+	+	+	+	+	+	+	+	15.0	+	98
	42	-	0	-	-	-	-	-	-	-	-	-	-	-	-	±	4
	43	0	0	±	±	-	-	±	+	+	+	+	+	+	± 1.3	+	7
	44	+	0	+	+	+	+	+	+	+	+	+	+	+	20.0	+	65
	45	-	0	-	-	-	-	-	-	-	-	±	-	-	-	±	3
*	46	-	0	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	47	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	48	0	0	-	-	-	-	-	-	-	-	-	-	-	-	±	4
	49	0	0	-	-	-	-	-	-	-	-	-	?-C ₂	-	-	-	à ref.
	50	-	0	+	±	+	-	±	+	+	-	+	-	-	+	± 1.3	±
*	51	-	0	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	52	-	0	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	53	-	0	-	±	-	-	-	-	-	-	-	-	-	± 1.3	+	9
	54	0	0	-	-	0	-	-	-	-	-	-	-	-	± 1.3	-	θ
	55	0	0	-	-	±	-	-	-	-	-	-	-	-	-	-	θ
*	56	0	0	+	+	+	+	+	+	±	+	+	+	+	2.0	+	12
	57	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	58	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	59	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	60	0	0	+	+	+	+	+	+	+	+	+	+	+	2.0	+	7
	61	0	0	+	+	+	+	+	+	+	+	+	+	+	2.5	+	23
	62	0	0	±	-	-	-	-	-	-	-	-	±	-	-	-	θ
	63	0	0	-	-	-	-	-	-	-	-	-	±	?-E.Fl.	-	-	θ
	64	0	0	±	±	±	-	-	-	±	-	-	?+C ₂	?-E.Fl.	-	-	θ
	65	0	0	-	-	-	-	-	-	-	-	-	-	-	-	±	4
*	66	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	67	0	0	+	+	+	+	+	+	+	+	+	+	+	35.0	+	55
	68	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	69	0	0	+	+	+	-	+	+	+	+	-	-	-	± 1.3	+	7
	70	0	0	-	-	-	-	-	-	-	-	-	-	-	-	±	4

* Examiné le 22/5. — Tested on 22/5.

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
			• t					•		Insuff. cordis Pyelitis Scarlatina Scarlatina	36 37 38 39 40	
		•	• t							Aortit. luica	41 42 43 44 45 46 47 48 49 50	
			• t		• t					Lupus vulg. Lymphom colli Malaria tertian.	51 52 53 54 55	
			• t							Scarlatina	56 57 58 59	
			• t	• t						Gon.	N. H. Syphilis 60	
		• t					•			Hemiplegia sin.	61 62 63 64 65	
•			• t				•	•		Dermatitis	66 67 68 69 70	
		•	• t						•	Lues cerebrospin. Melanosarcom		

Tableau 1. — Table. 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae			
								•			Cancer colli uteri Scarlatina	71	
									•			72	
							•					73	
							•					74	
							•					75	
							•					76	
							•					77	
							•					78	
							•			Pleuritis		= n° 836	79
							•					= n° 838	80
			• t									81	
			• t									82	
							•					83	
							•					84	
										Gon. Herpes gen.		85	
										Gon.		86	
			• t			• t						87	
												88	
				• t								89	
							•			Gon.		90	
							•					91	
		• t								Arthrit. luica		92	
			• t									93	
			•							Mb. cordis		94	
									•	Cancer ventr.		95	
							•	•				96	
							•					97	
							•		•	Cancer uteri		98	
							•					99	
			• t					•				100	
			• t									101	
			• t							Bronchitis diff.	Wien	102	
• t					• t							103	
		• t										104	
										Neuritis retrobulb.		105	

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernas R.
		De Biasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
22/5	106	—	0	—	+	±	—	—	±	+	—	±	—	—	+ 1.6	— 0
	107	—	0	±	+	+	—	—	+	±	—	±	—	+ 1.6	+	7
	108	—	0	±	—	+	—	—	+	±	+	±	E. Fl.	+ 2½	à ref.	
	109	—	0	—	+	±	—	—	+	±	—	—	—	—	±	5
	110	—	0	—	—	—	—	—	—	—	—	±	—	—	±	4
	111	—	0	+	+	±	—	+	±	±	±	+	±	±	5	+ 10
	112	—	0	—	±	+	—	—	±	+	+	±	—	—	3	± 4
	113	—	0	—	—	—	—	—	+	—	—	±	?-C ₂	—	± 1.3	à ref.
	114	—	0	—	—	—	—	—	—	—	—	—	?-C ₂	E. Fl.	—	à ref.
	115	—	0	±	±	±	±	—	±	+	—	±	—	—	+ 2	+ 11
	116	—	0	—	—	+	—	±	±	±	±	±	—	+	+ 1.6	± 4
	117	—	0	—	—	—	—	—	—	+	—	+	—	—	—	± 3
	118	±	0	±	±	±	±	+	±	±	±	±	+	+	+ 2	± 28
	119	—	0	+	—	+	—	±	±	±	±	±	+	±	± 1.3	+ 8
	120	—	0	—	—	—	—	—	—	—	—	—	—	—	± 1.3	± 3
	121	—	0	—	—	—	—	—	—	—	—	—	—	—	—	± 4
	122	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	123	—	0	—	—	—	—	—	—	—	—	—	—	—	—	± 3
	124	—	0	±	±	±	—	—	—	—	—	±	—	—	—	— 0
	125	—	0	—	—	—	—	—	—	—	—	—	—	—	—	à ref.
	126	—	0	±	±	±	—	—	±	+	±	±	±	0	+ 1.6	— 0
	127	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	128	—	0	—	—	—	—	—	—	—	±	—	?-C ₂	—	—	à ref.
	129	±	0	±	—	±	+	±	±	±	±	±	±	±	+ 2	± 20
	130	—	0	±	—	±	+	+	±	±	±	±	+	±	± 5	— 0
	131	—	0	—	—	—	—	—	±	+	—	+	±	—	—	— 0
	132	—	0	±	—	—	—	—	—	—	—	—	—	—	—	— 0
	133	—	0	±	±	±	—	+	±	±	±	±	±	±	± 5	± 12
	134	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	135	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	136	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	137	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	138	—	0	—	—	—	—	—	—	—	—	—	?-C ₂	—	—	— 0
	139	—	0	—	—	—	—	—	—	—	—	—	—	—	—	à ref.
	140	—	0	±	+	±	±	+	±	±	±	±	+	+	± 5	à ref.

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
		• t			• t						Wien	106
			• t									107
			• t									108
			• t									109
												110
			• t									111
			• t									112
•										Acne. vulg.		113
•												114
• t												115
			• t									116
					• t							117
					• t							118
					• t							119
								•				120
			• t					•		Eclampsia		121
										Adipositas		122
							•					123
										Colitis, Febrilia		124
										Melaena		125
			• t									126
								•				127
			• t					•				128
			• t									129
												130
			•							Lupus vulg.		131
			• t							M. Recklinghaus.		132
												133
										Lupus vulg.		134
										Lupus vulg.		135
									•	Cancer maxillae		136
									•	Cancer ventr.		137
							•			Pleuritis		138
									•	Cancer cordiae		139
			• t									140

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. 1. 2.	M. 2. 2.	M. 3. 2.	S. 2. 2.	S. 3. 2.	S. 4. 2.	S. 5. 2.
		De Blasi	Debalins	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn							
		Meinicke	Müller	Nagayo Nobuchi	Sachs Witselsky	Norel	Veres Lorel										
22/5	141	—	0	±	—	—	—	—	—	—	—	—	—	—	—	—	—
	142	±	0	+	+	+	+	+	+	+	+	+	+	+	5	+	22
	143	—	0	+	+	+	—	±	+	+	+	+	+	+	3	±	4
	144	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	145	+	0	+	+	+	+	+	+	+	+	+	+	+	125	+	80
	146	+	0	+	+	+	+	+	+	+	+	+	+	+	100	+	81
	147	+	0	+	+	+	+	+	+	+	+	+	+	+	45	+	30
	148	—	0	—	—	—	—	—	+	±	—	—	—	±	1.3	±	4
	149	—	0	±	—	±	—	—	—	—	—	—	—	—	—	—	—
	150	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	151	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	152	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	153	+	0	+	+	+	+	+	+	+	+	+	+	+	3	+	52
	154	—	0	—	—	±	—	—	—	—	—	—	—	—	—	—	—
	155	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
23/5	156	±	0	+	+	+	+	+	+	+	+	+	+	+	15	+	20
	157	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	158	—	0	+	+	+	+	+	+	+	+	+	+	+	25	+	23
	159	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	160	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	161	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	162	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	163	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	164	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	165	+	+	+	+	+	+	+	+	+	+	+	+	+	10	+	48
	166	—	+	+	+	+	+	+	+	+	+	+	+	+	1.6	—	—
	167	—	—	—	—	—	—	±	—	—	—	—	—	+	1.6	—	—
	168	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	169	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	170	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	171	—	—	—	—	—	—	—	—	—	—	—	—	±	1.3	—	—
	172	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	173	—	+	—	±	±	—	±	—	—	—	—	—	±	1.3	—	—
	174	+	+	+	+	+	+	+	+	+	+	+	+	+	5	à ref.	—
	175	—	±	—	+	±	—	±	—	±	—	±	—	+	1.6	—	—

Tableau 1. — Table 1.

Diagnosis										Observations	No.
Syphilita						Mb. alii (non-syphilit.)					
I	II	III	Latus	Cong.	Tabes	D. p.	The.	Grav.	Tumor	Diagn. Aliae	
•			• t								141
			• t							Eczema manus	142
			• t							Psoriasis	143
			• t								144
			• t								145
			• t								146
			• t								147
										Psoriasis	148
										Psoriasis	149
										Psoriasis	150
										Scabies	Berlin 151
										Ulcus cruris	152
			• t								153
										Gon.	154
										Psoriasis	155
			•								156
										Gon.	157
			•								158
										Gon.	159
										Gon.	160
										Mb. cordis	161
								• ?		Ulcus ventr.	162
								• ?		Cancer coli ?	163
										Dyspepsia	164
			• t								165
			• t								166
										Ulcus ad frenul.	= n° 798 167
										Lymphoma	168
										Lupus vulg.	169
										Lupus vulg.	170
			• t					•		Nephritis	171
								•			172
								•			173
			•								174
			•								175

Berlin

= n° 798

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Verna R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
$23/5$	176	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	177	—	—	—	+	—	—	—	—	—	+	—	—	—	—	—
	178	—	—	—	—	—	—	—	—	—	—	—	—	—	$+2\frac{1}{2}$	—
	179	—	+	+	+	+	+	+	+	+	+	+	+	+	± 1.1	± 5
	180	+	+	+	+	+	+	+	+	+	+	+	+	+	+	56
	181	—	—	—	—	—	—	—	—	—	—	—	—	E. Fl.	—	à ref.
	182	—	—	—	+	—	—	+	—	—	—	—	+	—	—	—
	183	+	—	+	+	+	+	+	+	+	+	+	+	+	10	6
	184	—	+	+	+	+	+	+	+	+	+	+	+	+	5	—
	185	—	—	—	+	—	—	—	+	+	—	— ¹	+	0	—	—
	186	—	+	—	+	+	+	+	+	+	+	+	+	+	5	± 3
	187	—	+	+	+	+	+	+	+	+	+	+	+	+	5	29
	188	—	à ref.	+	+	+	+	—	+	+	+	+	+	—	1.6	8
	189	+	+	+	+	+	+	+	+	+	+	+	+	+	10	à ref.
	190	+	+	+	+	+	+	+	+	+	+	+	+	+	35	130
	191	+	—	+	+	+	+	+	+	+	+	+	+	+	25	153
	192	—	—	—	—	+	—	—	—	—	—	+	?-C ₂	—	$+1.6$	—
	193	—	+	—	—	+	+	—	+	—	—	0	+	E. Fl.	—	6
	194	—	+	—	—	—	—	—	—	—	—	—	+	—	—	—
	195	—	à ref.	—	—	—	—	—	+	—	+	+	+	+	$+2\frac{1}{2}$	—
	196	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—
	197	+	+	+	+	+	+	+	+	+	+	+	+	+	25	132
	198	—	—	+	—	—	+	+	+	+	+	+	+	—	—	—
	199	—	—	+	+	+	+	+	+	+	+	+	+	+	1.6	à ref.
	200	—	—	+	+	+	+	+	+	+	+	+	+	+	3	8
	201	—	—	—	—	—	—	—	—	—	—	+	—	—	2	—
	202	—	+	—	—	+	—	—	—	—	—	+	—	—	1.6	—
	203	—	—	—	—	—	+	—	+	+	+	+	+	0	1.3	—
	204	—	—	+	—	—	+	+	+	+	—	+	+	—	1.6	—
	205	—	—	—	—	—	—	—	—	—	—	+	—	—	—	3
	206	+	+	+	+	+	+	+	+	+	+	+	+	+	5	45
	207	—	—	—	—	—	+	—	+	+	—	+	+	—	1.3	6
	208	—	—	+	+	+	+	+	+	+	+	+	+	+	2½	8
	209	—	+	+	+	+	—	—	+	+	+	+	—	+	3	4
	210	—	+	+	+	+	+	+	+	+	+	+	+	+	1.6	7

¹ Précipitation non spécifique. — Non specific precipitation.

Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit).						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae			
			• t				•		•	Lupus vulg. Prurigo atyp. Pleuritis exsud. Cancer colli uteri Lues cerebrospin.	= n° 837	176	
		• t							•			177	
			• t				•					178	
			•									179	
												180	
									•	Cancer recti		181	
	• t		• t				•					182	
			•									183	
												184	
							• ?			Pneumonia	Mortus: autopsia nulla	185	
			• t									186	
			•									187	
			•									188	
			•									189	
			•									190	
			•								Berlin	191	
			•							Gon.		= n° 862	192
										Gon.		= n° 863	193
										Gon.			194
									•			= n° 864	195
										Gon. Herpes		196	
			• t									197	
		• t										198	
			• t		• t							199	
												200	
		• t								Hemiplegia sin.	= n° 919	201	
			• t									202	
			• t									203	
					• t						Wien	204	
			• t									205	
• t													206
							• t						207
							• t						208
			• t									209	
						• t						210	

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

Date	No.	B. W. R.						Kahn R.		Mennicke	Muller	Nagaoka Isobach	Sachs Wiletsky	Sorel	Norel Lorenz
		Die Blasi	Debains	Harrison Wylea	Jacobsthal	Otto Blumenthal	Pavlovitch	Stenkowski	Boas						
24/5	246	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	247	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	248	-	±	-	-	-	-	-	-	-	-	2-C ₂	-	-	-
	249	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	250	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	251	±	à ref.	-	-	-	-	-	-	±	-	-	-	-	-
	252	-	-	-	-	±	±	-	-	±	-	-	-	-	-
	253	-	+	±	#	±	+	+	#	±	+	#	5.0	-	-
	254	±	-	-	-	-	±	-	-	-	2-C ₂	-	-	-	-
	255	±	# A ₅	#	#	#	+	+	#	#	#	#	+	2	+
	256	±	-	-	-	#	-	-	+	±	±	±	1.3	±	±
	257	±	# A ₄	±	#	#	#	±	±	±	±	+	1.6	±	±
	258	-	0	-	-	-	-	-	-	-	-	-	-	-	-
	259	-	0	-	-	-	-	-	-	-	-	±	-	-	-
	260	-	0	-	-	-	-	-	-	-	-	-	-	-	-
	261	±	0	+	+	#	+	#	#	#	#	#	#	1.5	#
	262	±	0	+	+	+	#	+	#	#	±	#	1.5	±	±
	263	±	0	#	#	#	#	#	+	#	#	#	#	2	#
	264	-	0	-	-	-	-	-	-	-	-	-	-	-	-
	265	-	0	-	-	-	-	-	-	-	-	-	-	-	-
	266	±	0	-	-	-	-	-	-	-	-	2-C ₂	-	-	-
	267	-	0	-	-	-	-	-	-	-	-	±	-	-	-
	268	-	0	-	-	-	-	-	-	-	-	-	-	-	-
	269	-	0	-	-	±	-	-	-	-	-	-	-	-	-
	270	-	0	#	#	#	#	#	±	#	-	+	±	2.2	+
	271	-	0	-	-	-	±	-	-	-	-	±	-	-	-
	272	#	0	#	#	#	#	#	#	-	#	±	#	2.5	±
	273	±	0	-	±	-	-	-	±	±	-	±	-	-	-
	274	-	0	-	±	-	-	-	-	-	-	±	+	1.6	±
	275	-	-	-	-	±	-	-	-	-	-	-	-	-	-
	276	±	0	#	#	#	#	#	#	+	#	+	#	1.5	+
	277	+	0	-	-	-	-	-	-	-	+	2-C ₂	-	1.5	±
	278	±	0	±	+	+	-	±	+	+	#	+	+	2.5	±
	279	+	0	-	-	-	-	-	-	-	-	±	-	-	-
	280	-	0	-	-	-	-	-	-	-	-	-	-	-	-

* Examiné le 24/5. ** Drs. Boas et Norel ont changé leurs antigènes.

* Tested on 24/5. ** Drs. Boas and Norel changed antigen.

Tableau 1. — Table 1.

Diagnosis										Observations	No.
Syphilis						Ml. all (non-syphilit.)					
I	II	III	4 5 6 7	8 9 10	11 12	13	14	15	16	Diagn. Abas.	
										Sang. cord. umbilic.	246
						•					247
								•			248
			•	t				•			249
			•	t							250
										Ulcus molle	251
								•			252
			•	t							253
			•	t							254
			•	t							255
			•	t							256
			•	t		•	t			Malaria tract.	257
			•	t							258
			•	t				•			259
			•	t							260
			•	t							261
			•	t							262
			•	t							263
			•	t							264
			•	t		•					265
			•	t							266
			•	t							267
										Dementia senil.	268
										Encephalit. epid.	269
						•	t			Malaria tract.	270
										Dementia alcohol.	271
						•	t				272
						•	t			Malaria tract.	273
		•	t							Lues cerebri	274
										Schizophrenie	275
						•	t			Malaria tract.	276
		•	t							Lues cerebrospin.	277
						•	t			Malaria tract.	278
										Dementia praecox	279
										Gon.	280
										N. H. Syph.	281
										Berlin	282

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
24/5	281	—	0	—	—	—	—	—	—	—	—	—	±	—	—	— 0
	282	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	283	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	284	+	0	—	—	—	—	—	—	—	—	±	—C ₂	—	—	— 0
	285	+	0	±	±	±	±	+	±	±	±	±	—C ₂	±	± 5	à ref.
	286	±	0	±	+	±	±	±	±	±	±	±	±	±	± 10	à ref.
	287	±	0	—	—	±	—	—	—	—	—	—	—	—	—	— 0
	288	±	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	289	—	0	—	—	—	—	—	—	—	—	±	—	—	—	± 4
	290	±	0	±	±	±	±	±	+	±	±	±	+	±	± 5	± 15
	291	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	292	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	293	—	0	—	—	—	—	—	—	—	±	—	—	—	—	— 0
	294	±	0	—	—	±	—	—	—	—	—	—	—	—	—	± 3
	295	—	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	296	±	0	—	±	—	—	—	—	—	—	—	—	—	± 1.3	à ref.
	297	+	0	±	+	±	—	±	±	±	+	+	+	±	± 2	± 3
	298	±	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	299	—	0	—	—	—	—	—	—	—	—	±	—	—	—	— 0
	300	—	+	±	+	±	±	±	±	±	±	±	±	±	± 25	± 54
	301	±	±	±	±	±	±	±	±	±	±	±	±	±	± 20	± 11
	302	±	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	303	±	0	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	304	—	± A ₅	±	±	±	±	±	±	±	±	±	±	±	± 2½	± 7
	305	±	± A ₃	±	±	±	±	+	+	±	±	±	+	+	± 2½	± 30
	306	—	—	—	—	—	—	—	—	—	—	—	—	—	± 1.3	± 3
	307	—	—	—	—	—	±	—	—	—	—	—	—	—	± 1.3	— 0
	308	—	± A ₄	+	+	±	±	±	±	±	±	0	+	+	± 2½	— 0
	309	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	310	—	—	—	±	±	—	—	—	—	—	±	±	—	—	— 0
26/5	311	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	312	—	—	—	—	—	—	—	—	—	—	—	—	—	± 1.3	— 0
	313	—	—	—	—	±	—	—	—	—	—	—	0	—	—	— 0
	314	—	—	—	—	—	±	—	—	—	—	—	0	—	—	— 0
	315	—	—	—	—	—	—	—	—	—	—	—	0	—	—	— 0

Tableau 1. — Table 1.

Diagnosis											Observations	No.		
Syphilis							Mb. alii (non-syphilit.)							
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae				
			● ^t ● ^t							Ulcus cruris	Berlin	281 282 283 284 285		
			● ^t										286	
							●		●	Dementia praecox		N. H. Syph.	287 288 289 290	
			● ^t ● ^t											291 292
										Encephal. epid. Eczema faciei Leucocytosis spin.			= n° 820	293 294 295
			● ^t							Hypertroph. prostat. Gon.	N. H. Syph.		296 297 298 299 300	
			● ^t ●											
			● ^t							Gon. Condylom. Alopecia areata		= n° 821	301 302 303 304 305	
						● ^t ● ^t								
						● ^t				Dementia paranoid. Dementia praecox Dementia senil.		Malaria tract.	306 307 308 309 310	
			● ^t							Dementia praecox. Epilepsia Dementia praecox Schizophrenia Schizophrenia	N. H. Syph.		311 312 313 314 315	

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witebsky	Norel	Vernes Bricq
25/5	351	+	+	A ₅	+	+	+	+	+	+	+	+	+	+	100	80
	352	+	+	A ₅	+	+	+	+	+	+	+	+	+	+	125	94
	353	±	—	—	—	—	—	—	—	—	—	?-C ₂	—	—	—	à ref.
	354	+	—	—	—	—	—	—	—	—	—	?+C ₂	—	—	—	à ref.
	355	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	356	+	+	A ₅	+	+	+	+	+	+	+	+	+	+	15	58
	357	+	+	A ₅	+	+	+	+	+	+	+	+	+	+	10	à ref.
	358	—	—	—	±	—	—	—	±	+	—	—	—	±	1.3	0
	359	±	Anti C.	—	—	—	—	—	—	—	—	—	—	—	—	0
	360	—	—	—	—	—	±	—	—	—	—	—	—	—	—	0
	361	±	—	—	—	—	—	—	—	—	—	±	—	—	—	0
	362	+	—	—	+	+	—	—	+	+	±	+	—	±	+ 2½	3
	363	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	364	±	Anti C.	—	—	—	—	—	—	—	—	—	—	—	—	0
	365	—	+	A ₄	—	+	—	—	—	—	—	—	—	—	—	0
	366	—	+	A ₃	—	+	+	—	±	+	+	+	+	+	+ 2½	3
	367	±	—	—	±	—	—	—	—	±	±	±	—	—	—	0
	368	—	—	—	±	+	—	—	—	±	—	±	±	±	+ 2½	0
	369	—	Anti C.	—	—	—	—	—	—	—	—	—	—	—	—	0
	370	—	+	A ₃	—	—	—	—	—	—	+	—	—	—	—	0
	371	—	—	—	—	—	—	—	+	+	+	+	+	+ 2½	+	11
	372	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	373	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	374	±	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	375	±	+	A ₃	±	±	±	+	+	+	+	+	+	+ 2½	+	7
	376	+	+	A ₃	±	±	—	±	+	—	—	+	+	±	1.3	0
	377	—	—	—	—	—	—	—	—	—	0	?-C ₂	—	—	—	0
	378	—	—	±	±	±	—	±	—	—	±	?-C ₂	—	—	—	à ref.
	379	—	—	—	—	—	0	—	—	—	—	±	—	—	—	0
	380	±	—	—	—	±	+	—	—	+	+	+	—	+	5	3
*	381	±	—	±	±	±	±	±	—	+	+	+	—	±	1.6	3
	382	—	—	—	—	—	—	—	—	+	—	+	—	±	1.3	4
	383	—	+	A ₄	±	±	+	—	—	+	+	+	+	±	5	0
	384	±	—	—	—	—	—	—	—	—	—	—	—	±	1.3	0
	385	±	—	—	—	—	+	—	—	±	±	±	—	+	1.6	3

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

Diagnosis											Observations	No.	
Syphilis							Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae			
										Gon.	N. H. Syph.	386	
										Gon.		387	
										Gon.		388	
										N.V.D.		389	
										Gon.		390	
										Gon.	N. H. Syph.	391	
										N.V.D.		392	
			• t									393	
										Gon.		394	
										Gon.		395	
										Gon.	London	396	
												397	
												398	
			• t									399	
										Gon.		400	
										Gon.		401	
										Ulcus ven.		402	
												403	
												404	
										Gon.		405	
										Gon.	Hamburg	406	
										N.V.D.		407	
										Gon.		408	
										Gon.		409	
										Gon.		410	
										Gon.		411	
												412	
										Gon.		413	
												414	
										Malaria tertian.		415	
											} London	416	
												417	
										Ulcus molle		Berlin	418
												B.-W. R. ‡ in clinic	419
										Gon.		London	420

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. I. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernas R.
		De Blasi	Debains	Harrison Wyer	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witebsky	Norel	Vernes Bricq
26/5	421	—	$\boxed{+A_2}$	—	—	\pm	—	—	—	—	—	—	—	—	—	θ
	422	\pm	—	—	—	—	—	—	—	—	—	—	—	—	—	4
	423	—	$\boxed{+A_3}$	\pm	\pm	\pm	—	—	—	—	—	—	—	—	—	θ
	424	—	Autl. G.	—	—	—	—	—	—	—	—	—	$\pm C_1$	—	—	θ
	425	—	—	—	\pm	—	—	—	—	—	—	—	—	—	—	θ
	426	—	$\pm A_3$	—	—	—	—	—	—	—	\pm	—	—	— \pm	± 1.3	± 3
	427	—	—	—	\pm	\pm	\pm	—	—	—	—	—	\pm	—	—	θ
	428	—	$\boxed{+A_3}$	—	—	—	—	—	—	—	—	—	—	—	—	θ
	429	\pm	$\pm A_3$	\pm	—	\pm	\pm	+	—	\pm	—	+	—	—	± 1.3	θ
	430	—	$\boxed{+A_4}$	\pm	$\boxed{+}$	$\boxed{+}$	—	—	—	—	—	—	—	—	—	± 3
	431	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	432	\pm	—	—	—	—	—	—	—	—	—	—	—	—	—	à ref.
	433	\pm	—	—	+	—	—	—	\pm	\pm	—	+	\pm	— \pm	± 1.3	θ
	434	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
	435	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
	436	—	—	—	\pm	—	—	—	—	—	—	—	—	—	—	± 3
	437	\pm	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
	438	\pm	—	\pm	\pm	—	—	—	—	—	—	—	— C_1	—	—	θ
	439	—	—	—	—	—	—	—	—	—	—	—	$\pm C_1$	—	—	θ
	440	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
	441	—	—	\pm	+	\pm	—	—	—	\pm	—	\pm	—	— \pm	—	θ
	442	—	—	\pm	\pm	\pm	—	—	—	—	—	—	—	—	—	θ
	443	—	—	—	\pm	\pm	—	—	—	—	—	—	—	—	—	θ
	444	—	—	—	—	—	—	—	—	—	—	—	—	—	± 1.3	θ
	445	—	—	—	—	—	—	—	—	—	—	—	—	—	—	θ
	446	—	—	—	—	—	—	—	—	—	—	—	?— C_2	—	—	θ
	447	—	—	—	—	—	—	—	—	0	—	—	—	—	± 1.3	0
	448	—	—	—	\pm	\pm	\pm	—	—	—	—	—	—	—	—	θ
	449	\pm	—	—	$\boxed{+}$	\pm	—	—	—	—	—	—	—	—	—	0
	450	—	—	—	—	\pm	—	—	—	—	—	—	$\pm C_1$	—	—	θ
	451	—	—	—	—	—	\pm	—	—	—	—	—	—	—	—	θ
	452	\pm	$\pm A_5$	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	± 100	± 81
	453	—	—	\pm	+	—	\pm	—	+	\pm	\pm	+	?+ C_2	—	± 1.3	3
	454	—	—	—	—	\pm	\pm	—	—	—	—	—	—	—	—	θ
	455	—	—	—	—	—	—	—	—	—	—	—	— C_1	—	—	θ

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
			• t							Gon.	N. H. Syph.	421
										Gon.	N. H. Syph.	422
										Gon.		423
										Gon.		424
										Gon.		425
		• t										426
										Gon.		427
			• t							Gon.	N. H. Syph.	428
										Gon.	= n° 957	429
										Gon.		430
										Gon.		431
					• t					Gon.		432
										Gon.		433
										Gon.		434
										Gon.	London	435
										Ulcus molle		436
										Gon.		437
										Gon.		438
										Gon.		439
• t												440
					• t							441
			• t									442
			• t									443
										Gon.		444
										Gon.		445
										Gon.		446
										Gon.		447
										Gon.		448
										Gon.	N. H. Syph.	449
	• t											450
												451
	•									Dement. senil.		452
		• t										453
			• t									454
										Psoriasis		455

Tableau 1. — Table 1.

Date	No.	B. W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
26/5	456	+	++ A ₃	++	++	++	++	++	++	++	++	++	++	++	++ 10	++ 13
	457	-	++ A ₃	-	-	++	-	-	±	+	++	++	+	±	++ 5	± 4
	458	±	++ A ₅	++	++	++	++	+	++	++	++	++	++	++	++ 25	++ 43
	459	-	-	-	-	-	±	-	-	-	-	-	- C ₁	-	-	0
	460	-	-	-	-	-	±	-	-	-	-	-	-	-	-	0
	461	-	-	-	-	-	+	-	-	-	-	-	?-C ₂	-	-	± 3
	462	±	-	-	-	-	+	±	-	-	-	-	-	-	-	0
	463	±	-	-	-	-	+	-	-	-	-	-	-	-	-	0
	464	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0
	465	-	++ A ₄	-	-	±	+	-	-	-	-	-	-	-	-	± 3
	466	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	467	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	468	-	-	-	-	±	-	-	-	-	-	-	-	-	-	0
	469	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	470	±	-	-	-	-	-	-	-	-	-	-	- C ₁	-	-	0
29/5	471	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	472	-	+ A ₂	±	+	+	-	-	++	++	++	+	?+C ₂	++	+ 2½	+ 6
	473	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	474	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	475	±	-	-	±	-	-	-	-	-	-	-	-	-	-	0
	476	-	-	-	-	-	-	-	-	-	-	-	-	-	-	± 5
	477	+	-	-	-	+	±	±	-	-	-	-	-	-	-	0
	478	-	-	-	-	-	-	-	-	-	-	-	-	-	-	± 4
	479	-	-	±	+	+	+	-	++	++	+	++	±	++	+ 2½	0
	480	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	481	-	-	-	±	-	-	-	++	++	-	+	±	- ++	-	0
	482	-	0	-	-	-	-	-	++	++	-	-	±	-	-	0
	483	-	++ A ₃	+	+	++	+	++	++	++	++	++	++	++	++ 5	+ 10
	484	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	485	-	-	-	+	-	±	±	++	++	-	+	±	- ++	± 1.3	0
	486	-	++ A ₃	-	±	++	-	-	++	++	++	+	+	- ++	++ 5	0
	487	-	-	-	+	±	-	-	-	-	-	-	-	-	-	0
	488	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	489	-	-	-	-	-	-	-	-	-	-	-	-	-	± 1.1	0
	490	-	-	-	-	±	-	-	-	-	+	-	-	±	-	0

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
					• t							456
			• t							Lues cerebrospin.		457
		• t					•			Neurasthenia		458
												459
												460
		• t										461
								•		Lupus vulg.	= n° 947	462
								•			= n° 949	463
								•			= n° 948	464
								•			= n° 946	465
										Lupus vulg.		466
								•				467
								•				468
										Lupus vulg.		469
							•					470
										Mb. cordis		471
		• t										472
										Lupus vulg.		473
										Lymphom. colli		474
								•				475
							•					476
								•		Eclampsia	N. H. Syph.	477
										Sang. cord. umbilic.		478
		• t										479
			• t					•				480
		•										481
										Encephal. luica		482
										Lupus vulg.		483
					• t					Hemiplegia dextr.		484
							•			Pleurit. tub.		485
			• t									486
			• t									487
			• t					•				488
			• t									489
							•				= n° 953	490

Tableau 1. — Table 1.

Tableau R.																		
Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. S. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.		
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witebsky	Norel	Vernes Briq		
29/5	491	-	-	-	-	-	-	-	-	-	-	-	+	-	-	0		
	492	-	-	-	±	-	-	±	-	-	-	-	-	-	-	0		
	493	+	# A ₄	#	#	#	#	#	#	#	#	#	#	#	# 10	# 57		
	494	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
	495	-	-	-	-	-	-	-	-	-	-	-	±	-	-	0		
	496	+	# A ₃	#	#	#	#	#	#	#	#	#	#	#	# 15	# 70		
	497	-	# A ₃	# ±	#	#	-	±	#	#	#	#	#	#	± 1.3	+	10	
	498	±	# A ₃	#	#	#	#	#	#	#	#	#	#	#	# 10	#	31	
	499	-	-	-	±	-	-	-	-	-	-	-	±	-	-	-	0	
	500	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	
	501	+	-	-	-	±	-	±	-	-	-	-	-	-	-	-	0	
	502	#	# A ₅	#	#	#	#	#	#	#	#	#	#	#	#	# 20	# 57	
	503	#	# A ₅	#	#	#	#	#	#	#	#	#	#	#	#	# 50	# 104	
	504	-	-	-	±	-	±	+	#	#	±	#	+	#	±	1.3	+	6
	505	±	# A ₃	+	±	#	#	#	#	#	#	#	#	#	#	# 10	+	9
	506	-	-	-	±	-	±	-	-	#	#	-	+	+	±	± 1.3	-	0
	507	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	508	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	509	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	510	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	0
	511	-	-	±	±	#	-	+	#	#	-	#	+	±	+	± 1.6	±	3
	512	-	0	#	#	#	+	#	#	#	#	#	#	-	#	± 2½	±	4
	513	-	# A ₃	+	-	±	-	±	#	#	-	+	±	-	#	± 1.3	±	3
	514	±	# A ₃	+	-	#	#	#	#	#	#	#	#	#	#	# 20	0	0
	515	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	0
516	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
517	-	# A ₄	-	-	+	-	-	-	-	-	-	-	-	-	-	-	0	
518	-	-	#	#	#	±	+	#	#	+	#	±	+	#	± 1.6	±	4	
519	-	-	#	#	#	#	#	#	#	#	#	#	#	+	± 2½	#	15	
520	-	0	±	±	-	-	-	-	-	-	-	-	?-C ₂	-	-	-	0	
521	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
522	-	-	±	+	-	-	±	±	-	-	-	-	-	-	-	0	0	
523	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
524	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
525	#	# A ₅	#	#	#	#	#	#	+	#	#	#	#	#	# 25	#	17	

Tableau 1. — Tab. 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murat R.	S. G. R.	Sigma R.	Vernas R.		
		De Biasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq		
29/5	526	+	+ A ₅	+	+	+	+	+	+	+	+	+	+	+	125	+	96	
	527	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
	528	-	-	-	±	±	-	-	+	+	+	+	+	±	1.3	+	31	
	529	+	+ A ₅	+	+	+	+	+	+	+	+	+	+	+	5	+	21	
	530	+	+ A ₅	+	+	+	+	+	+	+	+	+	+	+	5	+	7	
	531	-	-	-	-	±	-	-	+	+	+	+	+	+	1.3	±	4	
	532	-	+ A ₃	+	±	+	+	±	+	+	-	+	±	-	±	2½	±	5
	533	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
	534	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3	
	535	-	±	±	+	±	±	±	+	+	+	0	+	-	+	2½	±	4
	536	-	-	-	-	±	-	-	-	-	-	0	-	-	-	-	-	θ
	537	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	538	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	539	-	Anti compl.	-	-	-	-	-	-	-	-	-	-	-	-	-	±	4
	540	±	+ A ₂	-	+	+	±	-	-	-	-	±	+	±	±	1.3	±	4
	541	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	3
	542	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ
	543	±	-	+	+	+	+	+	+	+	+	+	+	+	+	5	à ref.	
	544	+	+ A ₃	+	+	+	+	+	+	+	+	+	+	+	+	10	+	20
	545	+	+ A ₅	+	+	+	+	+	+	+	+	+	+	+	+	25	+	54
546	-	+ A ₃	-	±	±	-	-	-	-	-	-	-	-	-	-	-	θ	
547	+	+ A ₅	+	+	+	+	+	+	+	+	+	+	+	+	25	+	81	
548	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
549	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
550	+	+ A ₅	+	+	+	+	+	+	±	±	+	+	+	+	2½	+	9	
551	+	+ A ₂	-	-	±	-	±	-	-	-	-	-	-	-	-	±	4	
552	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	θ	
553	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	θ	
554	-	-	+	+	+	+	+	+	+	+	+	+	+	+	10	+	15	
555	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	θ	
556	+	+ A ₂	+	+	+	+	+	+	+	+	+	+	+	+	10	à ref.		
557	±	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	θ	
558	+	-	+	+	±	±	±	+	+	+	±	+	+	+	5	+	13	
559	-	0	-	±	+	-	-	-	-	-	-	-	-	-	-	±	3	
560	-	-	±	-	±	-	-	+	+	+	+	+	+	+	1.6	-	θ	

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
	•									Ulcus molle		526
			• t									527
			• t									528
				• t								529
												530
			• t									531
			• t									532
			• t									533
							•				= n° 19	534
		•								Lues cerebrospin.	= „ 21	535
								•	•	Cancer uteri	= „ 72	536
								•	•	Cancer ventr.	= „ 95	537
											= „ 96	538
			• t							Eclampsia	= „ 121	539
											= „ 126	540
								•			= „ 127	541
									•	Cancer ventr.	= „ 137	542
			• t								= „ 229	543
			• t								= „ 235	544
			• t								= „ 236	545
			• t								= „ 237	546
			• t								= „ 241	547
			• t					•			= „ 248	548
						• t					= „ 249	549
											= „ 272	550
			• t							Dementia praecox	= „ 287	551
			• t							Gon.	= „ 299	552
			• t								= „ 302	553
			• t									554
			• t									555
			• t								Versailles	556
	• t									Aneurisma aortæ		557
										Hemiplegia sin.		558
	• t											559
												560

* Les mêmes sérums réexaminés. — The same sera re-tested.

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.		
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Muller	Nagayo Nobuchi	Sachs Witebsky	Norel	Vernes Bricq		
29/5	561	—	—	+	+	+	+	±	+	+	+	+	+	+	5	+	38	
	562	—	—	—	—	—	—	—	—	—	—	±	—	—	—	±	3	
	563	±	0	—	—	+	—	—	—	—	+	—	—	—	—	—	0	
	564	±	± A ₃	±	—	+	—	—	—	—	+	—	—	—	—	—	0	
	565	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	566	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	567	±	—	±	—	—	—	±	—	—	—	—	—	—	—	—	0	
	568	+	± A ₃	+	+	+	+	+	+	+	+	+	+	+	± 20	+	18	
	569	±	± A ₃	+	+	+	+	+	+	+	+	+	+	+	± 20	+	26	
	570	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	571	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	572	—	—	—	—	—	—	—	—	+	—	±	—	—	± 1.3	—	0	
	573	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	574	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	575	—	—	—	—	—	—	—	—	—	—	—	—	—	± 1.3	0	0	
30/5	576	±	—	+	+	+	+	+	+	+	+	+	+	+	± 10	+	15	
	577	+	—	+	+	+	+	+	+	+	+	+	+	+	± 10	+	34	
	578	—	± A ₂	—	—	±	—	—	+	+	+	+	+	± 1.3	—	0	0	
	579	±	± A ₅	+	+	+	+	±	+	+	+	+	+	± 15	+	50	0	
	580	—	—	—	—	—	±	—	±	—	+	—	—	± 1.3	—	0	0	
	581	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	
	582	—	± A ₃	+	+	+	+	±	+	+	+	+	+	±	± 5	±	4	
	583	—	± A ₃	±	±	+	+	—	+	+	+	+	+	E. Fl.	± 5	—	0	
	584	—	± A ₃	±	+	±	+	—	+	+	+	+	+	E. Fl.	± 2½	±	4	
	585	+	± A ₃	+	+	+	+	±	+	+	+	+	+	+	± 20	+	14	
	586	—	± A ₃	±	—	±	+	—	+	+	+	+	±	±	± 1.6	+	17	
	587	—	—	±	±	±	±	—	+	+	±	+	?	—	± 1.6	—	0	
	588	—	±	±	+	±	+	—	+	+	+	+	±	E. Fl.	± 2½	±	4	
	589	—	—	—	+	±	—	—	±	—	—	±	—	—	—	—	0	
	590	—	± A ₂	±	+	+	+	+	+	+	+	+	+	±	± 2½	+	9	
	591	+	± A ₃	+	+	+	+	±	+	+	+	+	+	?	± E. Fl.	± 5	+	26
	592	+	± A ₅	+	+	+	+	+	+	+	+	+	+	± E. Fl.	± 5	+	16	
	593	—	—	—	—	—	—	—	±	±	—	±	—	—	± 1.3	—	0	
	594	—	± A ₄	+	+	±	+	—	+	+	—	+	—	—	± 2	—	6	
	595	—	± A ₂	—	+	±	—	+	±	+	—	±	—	—	—	±	4	

Tableau 1. — Table 1.

Diagnosis										Observations	No.	
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D.p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
		• t								Observatio		561
			• t									562
			• t									563
			• t									564
			• t									565
			• t								Versailles	566
				• ?								567
			• t									568
			• t									569
			• t									570
							•			Pleuritis exsud. sin.		571
			• t				•					572
							•				Hamburg	573
							•		•			574
												575
					• c							576
			• t									577
			• t								Malaria tract.	578
			• t									579
		• t									Malaria tract.	580
			• t									581
			• t									582
			• t									583
			• t								Malaria tract.	584
		• t										585
											Wien	
												586
		• t										587
												588
		• t								Lues cerebri		589
			• t									590
		•										
		• t								Aortitis luica		591
												592
				• t								593
					•							594
			• t									595

Tableau 1. — Table 1.

[illegible]

[illegible]

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
			•							Gon.	} Berlin	631
			•									632
			•									633
			•									634
			•									635
			• t								} Wien	636
			• t									637
			• t									638
			• t									639
			• t									640
			• t									641
			• t									642
			• t									643
			• t									644
			•									645
			• t							Hemiplegia sin.	} Malaria tract.	646
• t			• t									647
			• t									648
			• t	• t								649
			•									650
			•							Paralysis agitans	} N. H. Syph.	651
							•			Angina pectoris		652
							•					653
												654
												655
										Mb. Hodgkin	} Hamburg	656
									•	Cancer recti		657
										Dementia praecox		658
			• t							Exhibitionismus		659
		• t										660
			• t							Imbecillitas	} 1900-15 Malaria	661
			• t							Psychos. man. depr.		662
			• t									663
						• t						664
										Paranoia		665

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis						Mb. alii (non-syphilit.)						
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						• t				Paranoia	Malaria tract.	666
						• t						667
			•									668
			•									669
												670
								•		Degen. psychopath. Dementia praecox Hemiplegia Trauma capitis Encephal. luica	N. H. Syph.	671
												672
												673
		•										674
												675
										Lupus vulg. Lupus erythem.		676
								•				677
		• t								Lues cerebrospin.	= n° 951	678
			• t									679
												680
										Schizophrenia Sang. cord. umbilic		681
						• t						682
		• t										683
			• t									684
												685
			• t							Scabies		686
												687
						• t						688
						• t						689
										Dementia praecox	Malaria tract.	690
						• t					N. H. Syph.	691
										Dementia praecox		692
						• t				Dementia praecox		693
												694
		• t										695
						• t						696
								•				697
												698
						• t						699
										Dementia praecox	N. H. Syph.	700

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. P. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyley	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
30/5	701	—	± A ₃	±	+	—	±	—	—	+	—	+	±	—	± 1.3	— 0
	702	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	703	±	± A ₅	±	±	±	±	±	—	±	—	±	±	—	± 10	± 71
	704	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	705	—	+ A ₂	±	±	±	±	+	±	±	+	±	±	±	± 1.3	0
	706	—	± A ₅	—	—	±	±	—	—	—	±	—	—	—	—	— 0
31/5	707	—	—	—	+	—	—	±	—	—	—	—	—	—	—	— 0
	708	±	± A ₅	±	±	±	±	±	±	±	±	±	±	±	± 25	± 75
31/5	709	—	± A ₄	±	±	±	±	±	±	±	±	±	±	+	+	0
	710	—	—	—	±	—	—	±	—	—	—	—	—	—	—	— 0
31/5	711	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	712	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
31/5	713	—	± A ₅	—	±	±	±	—	+	+	—	±	—	—	—	± 3
	714	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
31/5	715	—	—	—	±	—	±	±	—	—	—	—	—	—	—	— 0
	716	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	717	—	± A ₅	—	—	±	—	—	—	—	—	—	—	—	—	— 0
	718	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
31/5	719	—	—	+	±	±	±	+	±	±	±	+	+	—	± 1.3	— 0
	720	—	± A ₄	—	—	±	—	—	—	—	—	—	—	—	± 1.3	— 0
31/5	721	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	722	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	723	+	± A ₅	±	±	±	±	±	±	±	±	±	±	±	± 10	± 14
	724	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	725	±	± A ₅	±	±	—	±	—	±	±	—	±	—	—	± 1.3	— 0
	726	±	± A ₅	±	±	±	±	±	±	±	±	±	±	±	± 5	0
31/5	727	—	±	—	—	—	—	—	—	—	±	—	—	—	—	0
	728	±	—	—	+	+	—	—	±	—	—	—	—	—	—	— 0
31/5	729	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
	730	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— 0
31/5	731	—	± A ₄	+	±	±	±	±	±	±	±	±	±	±	± 5	± 15
	732	—	—	—	+	—	—	—	—	—	—	—	—	—	—	— 0
31/5	733	—	—	—	±	—	—	—	—	—	—	—	—	—	—	— 0
	734	+	± A ₅	±	±	+	±	—	±	±	+	+	+	±	± 2½	0
31/5	735	—	± A ₄	—	+	±	±	±	±	—	—	±	—	—	± 1.3	0

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. I. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernas R.
		De Blasi	Debalns	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel	Vernes Bricq
31/5	736	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	737	—	0	—	—	±	—	—	—	—	—	—	—	—	—	—
	738	±	± A ₄	±	±	±	±	±	±	±	±	±	+	±	± 10	± 20
	739	—	0	—	—	—	—	—	—	—	—	—	—	—	—	0
	740	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	741	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	742	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	743	—	± A ₄	±	±	±	±	+	±	±	±	±	±	±	± 2½	± 27
	744	—	0	—	—	+	—	—	±	—	+	—	? - C ₂	±	± 1.3	0
	745	—	—	—	±	—	—	±	—	—	—	—	—	—	—	0
	746	—	0	—	—	—	—	—	—	—	—	—	±	—	—	0
	747	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	748	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	749	±	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	750	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	751	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	752	—	± A ₄	—	±	+	+	±	±	+	±	±	±	—	± 1.3	0
	753	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	754	—	—	—	+	+	—	—	—	—	—	—	—	—	—	0
	755	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	756	±	—	—	+	—	—	—	—	—	—	—	—	—	—	0
	757	±	—	—	—	—	—	—	—	—	—	—	±	—	—	0
	758	+	± A ₄	±	±	±	±	±	±	±	±	±	±	±	± 15	0
	759	—	—	—	+	±	—	—	—	—	—	—	—	—	—	—
	760	±	± A ₄	±	±	±	±	±	±	±	±	±	±	±	± 25	0
	761	—	0	—	—	—	—	—	—	—	—	—	—	—	—	0
	762	—	—	—	±	—	—	—	—	—	—	—	—	—	—	0
	763	+	± A ₄	±	±	±	±	+	±	±	±	±	±	±	± 10	± 29
	764	±	± A ₅	±	±	±	+	±	±	+	+	+	+	±	± 5	0
	765	—	—	—	—	—	±	—	—	—	—	—	—	—	—	—
	766	—	0	±	±	—	—	±	+	±	±	+	+	—	± 1.3	0
	767	—	0	—	—	—	—	—	—	—	—	—	—	—	—	0
	768	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	769	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	770	—	± A ₅	±	±	±	—	±	±	±	+	±	±	+	± 2½	0

Diagnosis										Observations	No.
Syphilis						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor		
						• t		•		Prurigo atyp.	736 737 738 739 740
			• t							Observ.	741 742 743 744 745
			• t • t • t							Dement. praecox	746 747 748 749 750
										Gon.	N. H. Syph.
										Gon.	751 752 753 754 755
										Gon.	[Syph.]
										Gon.	London N. H.
			• t								756 757 758 759 760
			• t • t • t								761 762 763 764 765
										Gon.	766 767 768 769 770
										Gon.	Hamburg

Tableau 1. — Table 1.

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murela R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nolechi	Sachs Witebsky	Norel	Vernes Eriq
31/5	806	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	807	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	808	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	809	—	—	—	—	±	—	—	—	—	—	—	—	—	—	0
	810	±	0	—	—	±	—	—	—	—	—	—	±	—	—	0
	811	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	812	±	0	±	±	±	±	+	±	±	±	±	±	±	± 10	0
	813	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	814	—	± A ₄	—	±	±	—	—	—	—	+	—	—	0	—	0
	815	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—
	816	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	817	—	0	—	—	—	—	—	—	—	—	—	—	0	—	—
	818	—	± A ₃	—	—	—	—	—	—	—	—	—	—	—	—	—
	819	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	820	±	± A ₅	—	—	±	±	—	—	—	—	—	—	—	—	0
	821	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—
	822	±	—	—	+	+	—	—	—	—	—	—	—	—	—	—
	823	—	± A ₅	±	+	±	±	—	—	—	+	—	—	—	—	—
	824	+	0	±	±	±	±	+	±	±	±	±	±	±	± 50	± 72
	825	±	± A ₅	±	±	±	±	±	±	±	±	+	±	±	± 50	± 59
	826	—	± A ₂	±	±	±	±	—	±	±	+	+	+	±	± 2½	—
	827	±	± A ₄	±	±	±	±	±	±	0	±	±	±	—	± 25	± 39
	828	—	—	—	±	—	—	—	—	—	—	—	—	—	—	0
	829	—	± A ₃	—	—	—	—	—	—	—	—	—	—	—	—	—
	830	±	0	—	+	+	—	—	—	—	—	—	—	—	—	0
	831	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	832	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	833	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	834	±	0	±	±	±	±	±	—	±	±	—*	+	±	± 2½	± 6
	835	±	0	—	±	—	±	—	—	±	—	—	+	±	± 1.3	0
1/6	836	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	837	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
	838	—	0	±	±	±	±	±	±	±	±	±	—	E. Fl.	± 2½	0
	839	—	0	—	±	—	—	—	—	—	—	—	—	—	—	—
	840	—	0	±	±	+	—	+	±	0	+	+	—	±	± 1.3	0

* Température de l'incubateur : 33° C. — Temperature of incubator : 33° C.

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	G av.	Tumor	Diagn. Aliae		
			• t				•			Pleuritis exsud.		806
			• t				•			Pleuritis exsud.		807
			• t									808
			• t									809
												810
			• t					•				811
								•				812
								•				813
								•			N. H. Syph.	814
								•				815
								•				816
								•				817
								•			N. H. Syph.	818
										Angina Vincenti		819
										Eczema faciei	cf. n° 294; N. H. Syph.	820
										Gon. Condylom.	cf. n° 302; N. H. Syph.	821
			•								cf. n° 4	822
			• t									823
			•									824
			• t									825
						•						826
						• t						827
										Dementia praecox		828
			• t							Encephalit. epid.	N. H. Syph.	829
										Lues cerebrospin.		830
										Dementia praecox		831
										Paranoia		832
										Depressio ment.		833
			• t			•					Malaria tract.	834
												835
							•			Pleurit. exsud. sin.	cf. n° 79; N. H. Syph.	836
							•			Pleurit. exsud.	cf. n° 178; N. H. Syph.	837
							•				cf. n° 80; N. H. Syph.	838
										Encephalit. epid.	N. H. Syph.	839
			•							Paranoia		840

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						• t				Dementia praecox	N. H. Syph.	841
										Dementia praecox	} Hamburg	842
										Kachexia sen.		843
										Dementia praecox		844
												845
						• t				Schizophrenia		846
						• t				Kachexia sen.	Hamburg	847
						• t						848
						• t						849
												850
						• t				Dementia praecox	N. H. Syph.	851
						• t				Dementia praecox		852
						• t						853
						• t						854
												855
	• t					• t						856
												857
							•			Dementia alcohol.		858
							•				N. H. Syph.	859
											N. H. Syph.	860
							•				Berlin	861
			•							Gon.	cf. n° 192; N. H. Syph.	862
											cf. n° 193;	863
									•		cf. n° 195; N. H. Syph.	864
										Degenerat. psych.		865
										Encephalopath. art.		866
								•			= n° 955	867
										Epilepsia	N. H. Syph.	868
								•		Bronch. acut.	cf. n° 10; N. H. Syph.	869
										Dementia praecox	N. H. Syph.	870
						• t						871
						• t				Dementia praecox	N. H. Syph.	872
												873
									• ?	Melaena	N. H. Syph.	874
										Observatio		875

Tableau 1. — Table 1.

[illegible]

Tableau 1. — Table 1.

Diagnosis											Observations	No.
Syphilis							Mb. alli (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc	Grav.	Tumor	Diagn. Aliae		
			• ^t					•		Ulcus plantae		911
			•									912
										Psychos. man. depr.		913
		• ^t										914
												915
			• ^t							Malaria tropic.	Hamburg	916
			• ^t									917
			• ^t									918
		•								Hemipleg. sin.	cf. n° 201	919
										Observatio		920
					• ^t						Malaria tract.	921
			• ^t									922
					• ^t							923
			• ^t									924
			• ^t									925
• ^t			• ^t								Wien	926
			• ^t									927
			• ^t									928
			• ^t									929
			• ^t									930
					• ^t							931
			• ^t									932
			• ^t									933
		• ^t										934
												935
		•								Uraemia	Hamburg	936
												937
								•				938
								•			N. H. Syph.	939
								•				940
								•				941
								•				942
										Malaria tropic.	N. H. Syph.	943
			•?							Malaria tropic.	Hamburg	944
												945
								•			cf. n° 500; N. H. Syph.	945

Tableau 1. — Table 1.

Date	No.	B.-W. R.						Kahn R.		M. T. R.	M. S. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobechi	Sachs Witebsky	Norel
$\frac{2}{6}$	946			—		—				—				—	
	947			—		—				—				± 1.3	
	948			—		—				—				—	
	949			—		—				—				—	
	950			—		—				—				—	
	951			—		+			—	±				± 1.3	
	952			±		—			±	+	+			+	2
	953			±		+			—	+	+			—	
	954			—		0			—	—	—			—	
	955			—		0			—	—	—			—	
	956			±		0			—	—	—			—	
	957			±		0			—	—	—			—	

Tableau

RÉACTIONS POSITIVES DANS LES CAS QUI N'ONT PAS ÉTÉ
POSITIVE REACTIONS IN CASES NOT

Les sérums qui ont été
Sera which have been

Date	No.	B.-W. R.							Kahn R.		M. T. R.	M. B. R.	Murata R.	S. G. R.	Sigma R.	Vernes R.
		De Blasi	Debains	Harrison Wyler	Jacobsthal	Otto Blumenthal	Pavlovitch	Sierakowski	Boas	Kahn	Meinicke	Müller	Nagayo Nobecki	Sachs Witelsky	Norel	Vernes Bricq
21/5	10	0	0	—	—	+	—	—	—	—	±	—	—	—	—	—
1/6	869	—	0	—	+	+	—	—	—	—	—	—	—	—	—	—
22/5	53	—	0	—	±	—	—	—	—	—	—	—	—	—	± 1.3	+
22/5	79	—	0	—	—	—	—	—	—	—	—	—	?-C ₂	—	+ 1.6	à re
31/5	836	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22/5	148	—	0	—	—	—	—	—	+	±	—	—	±	—	± 1.3	±
23/5	167	—	—	—	—	—	—	±	—	—	—	—	—	—	+ 1.6	—
31/5	798	—	0	—	+	+	—	—	—	—	—	—	—	—	—	—
22/5	173	—	+	—	±	±	—	±	—	—	—	—	—	—	± 1.3	—
23/5	178	—	—	—	—	—	—	—	—	—	—	—	—	—	+ 2½	—
1/6	837	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—
23/5	185	—	—	—	+	—	—	—	±	±	—	—	±	0	—	—
23/5	192	—	—	—	—	±	—	—	—	—	—	+	?-C ₂	—	+ 1.6	—
1/6	862	—	0	—	—	+	±	—	—	—	—	±	—	—	—	0
23/5	195	—	à ref.	—	—	—	—	—	±	—	+	±	+	±	+ 2½	—
1/6	864	—	0	—	—	—	—	—	±	±	±	±	?-C ₂	—	± 1.3	0
24/5	230	—	+	—	±	±	—	—	—	—	±	—	—	—	—	—

Table 2.

DIAGNOSTIQUES CLINIQUEMENT COMME « SYPHILIS ».
DIAGNOSED CLINICALLY AS SYPHILIS.

xaminés sont groupés ensemble.

e-tested are grouped together.

Diagnosis										Observations	No.
Syphilis						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor		
								●		Bronchit. acuta	10 869
										Malaria tertian.	53
							●			Pleurit. exsud.	79 836
										Psoriasis	148
										Ulcus ad fren.	167 798
								●			173
							●			Pleurit. exsud.	178 837
							●?			Pneumonia	185
										Gon.	192 862
								●		C. maxil. sup.	195 864
										Lupus vulg.	230

Tableau 2. — Table 2.

[illegible]

Tableau 2. — Table 2.

[illegible]

Tableau 2. — Table 2.

Diagnosis										Observations	No.
Syphilis						Mb. alli (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor		
										Gon.	421
										Gon.	423
										Gon.	428
										Gon.	430 957
										Gon.	449
								•			462 947
										Lupus vulg.	463 949
								•			464 948
								•			465 946
								•		Eclampsia	477
								•			500 945
								•			501
										Hemiplegia sin.	559
										Ulcus molle	612
										Gon.	615
										Cholecystitis	626
										Gon.	627
						•					654

Tableau 2. — Table 2.

[illegible]

Tableau 2. — Table 2.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D.p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
										Dementia praecox		658
								•		Degen. psychopath.	¹⁷ / ₁₆ WR —, Kahn —, Σ ⁺ , M.T.R. —	671
							•					678
												951
										Dementia praecox	¹¹ / ₁₆ Sigma —, WR —, M.T.R. —	690
										Dementia praecox	Malaria tr.	700
								•				706
								•				707
								•				717
										Gon.		744
										Gon.		754
										Gon.		756
										Lupus vulg.		786
							•					796
								•				814
								•				818
										Encephalit. epid.	¹⁷ / ₁₆ WR —, Kahn —, Σ —, M.T.R. —	829
										Encephalit. epid.		839
										Dementia praecox		841
										Dementia praecox		851
							•					859

Tableau 2. — Table 2.

Diagnosis										Observations	No.
Syphillis						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae	
							•				860
								•			867
											955
										Epilepsia ?	¹⁵ / ₁₆ WR —, Kahn —, Σ —, M.T.R. — 868
										Dementia praecox	870
										Dementia praecox	¹⁵ / ₁₆ WR —, Kahn —, Σ —, M.T.R. — 872
									• ?	Melaena	874
											954
										Kachexia senil.	880
										Acne vulg. faciei	883
										Morbilli	902
											956
								•			939
										Malaria trop.	943

Tableau 3.

LIQUIDES CÉPHALO-
CEREBRO-SPINAL

Date	No.	B.-W. R.			Kahn R.	M. T. R.	M. B. R.	S. G. R.	Sigma R.	Vernes R.
		Harrison Wyler	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	Norel	Vernes Bricq
23/5	1	+	+	+	+	+	+	+	+ 3	126 ¹
	2	+	+	+	+	-	+	-	+ 1.6	48
	3	+	-	-	+	-	+	-	+ 1.6	30
	4	+	+	+	-	+	+	+	+ 2½	0 ¹
	5	+	+	+	+	+	+	E. Fl.	+ 2½	147 ¹
	6	+	+	+	+	-	+	-	+ 2½	12
	7	-	-	-	-	-	-	-	+ 1.3	8
	8	-	-	-	-	-	-	E. Fl.	-	7
	9	+	+	+	+	+	+	E. Fl.	+ 3	141
	10	+	+	+	+	+	+	E. Fl.	+ 2½	1
	11	-	-	-	-	-	-	-	- 0.9	7
	12	+	-	-	-	-	-	-	+ 1.3	129 ¹
	13	-	-	-	-	-	-	-	-	11 ¹
	14	-	-	-	-	-	-	-	-	8 ¹
	15	-	-	-	-	-	-	-	-	7
24/5 ²	16	+	0	0	0	0	0	0	-	114 ¹
	17	+	-	-	-	-	-	-	+ 2½	36 ¹
	18	-	-	-	-	-	-	-	-	5
	19	+	-	-	-	+	-	-	+ 1.6	119
	20	0	-	-	-	+	-	-	-	2
	21	-	-	-	-	-	-	-	-	0
	22	-	-	-	-	-	-	-	-	119
	23	-	-	-	-	-	-	-	-	9
	24	-	-	-	-	-	-	-	-	4
	25	-	-	-	-	-	-	-	-	6
	26	-	0	0	0	0	0	0	-	8
	27	-	-	-	-	-	-	-	-	9
	28	0	-	-	-	-	-	-	-	7
	29	-	-	-	-	-	-	-	-	5
	30	-	-	-	-	-	-	-	-	8

¹ Liquores contamin. decompos. ² Examinés le 29/5. — Tested on 29/5.

BRACHIDIENS.
FLUIDS.

Diagnosis										Observations	No.
Syphilis						Mb. alii (non-syphilit.)					
I	I	III	Latens	Cong.	Tabes	D.p.	Tbc.	Grav.	Tumor	Diagn. Aliae	
						● ^t ● ^t ● ^t ● ^t ● ^t					
					●	●				Schizophrenia	
					●	● ●					
			● ^t ● ^t ● ^t ● ^t ● ^t								
			● ^t		● ^t						
			● ^t			● ^t				Paraplegia	
					● ^t						
			● ^t ●		● ^t						
			● [?]		● ^t					Herpes	
			●								
			● ^t ● ^t ● ^t		● ^t						

Tableau 3. — Table 3.

[illegible]

Tableau 3. — Table 3.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						•				Paraplegia Uraemia	} Paris	31
						• t				Arteriosclerosis		32
						• t						33
						• t				Dementia praecox		34
						• t				Encephalit. epid. Dementia senil.		35
						• t					Malaria tract.	36
						• t						37
						• t						38
						• t						39
						• t						40
						• t				Dementia praecox Dementia alcohol.	Malaria tract.	41
						• t						42
						• t						43
						• t				Observatio		44
						•						45
			•				•					46
										Arterioscl. cerebri Parkinsonismus		47
					• t							48
			• t									49
			• t									50
		• t								Dementia praecox	} Paris	51
			• t									52
			• t									53
			• t							Arterioscl. cerebri		54
												55
										Observatio Delirium alcohol. Bronchit. chr. Dementia praecox Debilitas mental.	Observ.	56
												57
												58
												59
												60
										Dementia praecox Dementia senil. Arterioscl. cerebri		61
												62
												63
						•						64
			• t									65

Tableau 3. — Table 3.

Date	No.	B.-W. R.			Kahn R.	M. T. R.	M. S. R.	S. G. R.	Sigma R.	Vernes R.
		Harrison Wyler	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	N el	Vernes Briq
25/5	66	+	—	±	—	—	+	—	— 0.9	± 3
	67	—	—	—	—	—	0	E. Fl.	—	± 1
	68	+	+	+	+	+	+	+	— 0.9	± 114
	69	—	—	—	—	+	—	—	— 0.9	— 0
	70	+	—	—	±	±	+	—	— 0.9	± 2
	71	+	+	+	+	+	+	+	+ 2½	± 77
	72	+	+	+	+	+	+	+	— 0.9	± 126
	73	+	+	+	+	+	+	+	± 5	± 136
	74	+	+	+	±	—	±	E. Fl.	—	— 0
	75	—	—	—	0	—	—	E. Fl.	—	0
	76	—	—	—	—	—	—	—	—	± 2
	77	—	—	—	—	—	±	—	—	0
	78	+	—	—	±	—	+	±	—	+ 7
	79	—	—	—	—	—	—	—	—	— 0
	80	+	+	+	±	+	+	±	+ 1.6	± 5
	81	+	+	+	+	+	0	+	+ 2½	± 70
	82	+	+	+	+	+	+	+	± 5	± 132
	83	+	+	+	+	+	+	+	+ 2½	± 127
	84	+	+	+	+	+	+	+	+ 2½	± 125
	85	+	+	+	+	+	+	—	—	0
29/5	86	+	0	0	0	±	+	0	+ 2½	± 114 ¹
	87	+	—	—	—	—	±	0	—	± 12 ¹
	88	+	—	—	—	+	+	—	+ 1.6	± 141 ¹
	89	—	—	—	—	—	—	—	—	± 3 ¹
	90	—	—	—	—	—	± ¹	—	—	+ 10 ¹
2	91	—	—	—	—	—	—	—	—	± 5 ¹
	92	+	—	—	+	—	+	—	—	± 49 ¹
	93	+	0	0	+	+	+	0	+ 2½	± 134 ¹
	94	+	—	—	0	+	+	—	+ 2½	± 96 ¹
	95	+	—	—	—	+	+	—	+ 2½	± 124 ¹
	96	+	—	—	—	+	+	—	± 5	± 119 ¹
	97	±	—	—	—	—	+	—	—	— 0 ¹
	98	—	—	—	—	—	—	—	—	— 0
	99	+	+	+	+	±	—	—	—	— 0
	100	+	+	+	+	+	+	—	+ 1.6	± 104

¹ Examinés avec une quantité de liquide insuffisante (1,2 et 0,8 cm³ au lieu de 1,60 cm³). — Tested with too small quantity of fluid (1.2 and 0.8 cc. instead of 1.60 cc.).

² Examinés le 30/5. — Tested on 30/5.

Tableau 3. — Table 3.

Diagnosis											Observations	No.
Syphilis							Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor	Diagn. Aliae		
						• t						66
						• t						67
						• t						68
						• t						69
						• t						70
						• t						71
						• t						72
						• t						73
						• t						74
										Dementia alcohol.		75
		•								Mb. Alzheimer.		76
						• t				Lues cerebri		77
						• t				Malaria tract. 1926		78
						• t				„ „ 1925		79
						• t				„ „ 1925		80
						•				Berlin		81
						•						82
						•						83
						•						84
						•						85
					• t							86
					• t							87
					• t	• t						88
					• t							89
					• t							90
	• t									Lues cerebri		91
						• t					Wien	92
						•						93
						• t						94
						• t						95
						• t				Malaria tract.		96
					• t							97
					• t							98
					• t							99
					• t							100

Tableau 3. — Table 3.

Date	No.	B.-W. R.			Kahn R.	M. I. R.	M. B. R.	S. G. R.	Sigma R.	Vernas R.
		Harrison Wyer	Jacobsthal	Otto Blumenthal	Kahn	Meinicke	Müller	Sachs Witebsky	Norel	Vernes Brice
29/5	101	+	+	+	+	+	+	+	5	119
	102	±	+	±	±	—	—	0	—	θ
	103	+	+	+	+	+	+	+	1.6	123
	104	—	—	—	—	—	—	—	—	θ
	105	+	+	+	+	+	+	+	1.6	137
	106	—	—	—	—	—	—	—	—	θ
	107	—	—	—	—	—	—	—	—	θ
	108	+	+	+	+	+	+	+	0.9	146
	109	+	+	+	+	—	+	—	2	136
	110	+	+	+	+	+	+	+	15	136
	111	+	+	±	+	—	+	—	—	θ
	112	—	—	—	±	—	±	—	—	θ
	113	+	+	+	+	+	+	+	1.6	113
	114	+	+	+	+	+	+	±	2½	133
	115	+	+	+	+	—	+	—	0.9	65
30/5	116	—	—	—	—	—	—	—	—	± 5 ¹
	117	—	—	—	—	±	—	—	—	θ
	118	+	+	+	+	+	+	+	2½	124
	119	+	+	+	+	—	+	—	0.9	34
	120	+	+	+	+	+	+	—	2½	116
	121	—	—	—	—	—	—	—	—	θ
	122	+	+	+	+	+	+	+	2½	112

¹ Examinés avec une quantité de liquide insuffisante (1,2 et 0,8 cm³ au lieu de 1,60 cm³). — Tested with too small quantity of fluid (1.2 and 0.8 cc. instead of 1.60 cc.).

Diagnosis										Observations	No.
Syphills						Mb. alii (non-syphilit.)					
I	II	III	Latens	Cong.	Tabes	D. p.	Tbc.	Grav.	Tumor		
						● t				Encephalit. epid.	101
						● t					102
						● t				Dementia alcohol.	103
						● t					104
											105
										Encephalit. epid.	106
										Schizophrenia	107
						● t					108
						● t					109
						● t					110
						● t					111
						● t					112
						● t					113
		●								Lues cerebrospin.	114
						● t					115
										Dementia praecox	116
										Dementia praecox	117
						● t					118
						● t					119
						● t					120
										Psychos. man. depr.	121
						● t					122

OBSERVATIONS ON THE RESULTS OBTAINED.

PROFESSOR D. DE BLASI.

Concerning the Results on May 30th.

Not having been able, as is usually the case, to make a second test after a convenient interval, I have given the results of the first test, immediately after having taken the tubes from the thermostat. This must be borne in mind in view of the fact that some of the results, though strongly positive during the first test, can be due to a simple delay of hemolysis.

DR. E. DEBAINS.

Like all unheated-serum methods, our technique requires fresh sera, *i.e.*, sera taken twenty-four or forty-eight hours before examination. These are the normal conditions of work in the dispensaries, institutes and hospitals in the Paris area.

The Conference was working simultaneously on very fresh sera and sera of varying ages, up to nine days in certain cases (sera Nos. 554-569). We were obliged to increase the customary doses of alexin required for super-activation, and there was consequently an excess of alexin for the fresh sera and a shortage for the older sera; in the case of the latter, it was often difficult to read the results. These anomalies led in a number of cases to discrepancies as regards both sensitivity and specificity; this is what we found when we compared the results we obtained before the Conference with those obtained in the course of its work with sera from patients personally known to us which were sent to Copenhagen by aerial post on our instructions. This instance clearly illustrates our observations.

As nobody but ourselves uses a fresh-serum method, we were at a disadvantage, and our results are not strictly comparable with those of our colleagues. In spite of this unsatisfactory position, an examination of the results obtained will show the future importance of a comparison between our method, applied in normal conditions, and, in particular, the flocculation tests, in the detection of hereditary syphilis and latent or unrecognised syphilis in general medicine and surgery.

OBSERVATIONS BY DR. E. JACOBSTHAL.

The Serum Conference at Copenhagen yielded a surprising result, since it cannot be denied that the testing of the serum — not of the fluid — by means of complement-fixation gave less constant and less reliable results than the precipitation methods. It must, however, be pointed out that a research worker experimenting in a laboratory to which he is not accustomed is far less favourably situated than he would be in his own laboratory. Thus, at Hamburg for instance, the complement is never used only a few hours after collection, but is allowed to stand about twenty-four hours until it has acquired a certain consistency, while the sheep's blood is used in a far less concentrated form. Further, according to the unanimous opinion of the research workers who employed complement-fixing methods, the reagents supplied were, for some

unknown reason, less satisfactory than usual on certain days, especially the last days. If one considers, for example, the results of Jacobsthal's experiments, one finds that, while on the last three days of the experiments a large number of non-specific results were suddenly obtained, there were practically no such results during the first days of the Conference. (A confusion, no longer explicable now, must have arisen in the case of serum 185, which is a distinct exception to this rule and which gave a ++ result in Jacobsthal's experiments with all, even the weakest reactions.) The above consideration, moreover, shows that the precipitation methods are, as it were, less sensitive as far as changes of the local conditions are concerned, which must be considered a material advantage. It is only very seldom that we (Jacobsthal) obtain non-specific results in our own laboratory. The extract I used in Copenhagen appeared, for reasons unknown to me, to have reacted far more strongly than usual. At home such an occurrence is always counteracted by the clinic, and above all by the parallel use of precipitation methods. This, however, was not allowed at Copenhagen.

It must be pointed out in particular that, on the last day of the experiments, June 1st, 16 non-specific positive reactions were suddenly obtained. If that day's results were disregarded — in practice the results of such a "day of non-specific positivity" would always have been automatically eliminated — the Jacobsthal methodology would appear in a more favourable light, especially in view of its great sensitiveness in the indisputable cases of syphilis.

PROFESSOR R. MÜLLER.

I should like to make the following observations concerning our own M.B.R. results (clotting test). In non-syphilitic cases we obtained 7 doubtful = \pm reactions and one + reaction. Of these 8 reactions, 3 were repeated. One of the 3 cases was the + case which gave a \pm result on retesting. The results of the other 2 \pm cases were unchanged when retested. We think that the results of this retest should be reckoned among the successes of the M.B.R., as it points to the constancy of the reaction, and the value of \pm tests in cases of syphilis can be determined accordingly, since they may probably be construed as genuine and not merely accidental \pm reactions. Only the one non-syphilitic + case became a \pm case, which after all should also be counted as a success. Instead of this, in the final results, these 3 \pm retest reactions are classed separately as non-specific results, and by this classification, in the clotting reaction, the results for non-syphilitic cases are given as one + and 10 \pm reactions, instead of one + and 7 \pm . This point might also be taken into account in the case of the other persons concerned in these researches.

Dr. K. NOREL.

The extract for the Sigma reaction used during the first two days of the Conference gave a flocculation of quite a different type from the usual; it was therefore abandoned and a fresh preparation was used for the rest of the tests. It might be of interest to draw attention to the fact that five out of six non-specific weak positives (1.6 to 2½ units per cc.) obtained during the Conference were found with precisely this non-typical reacting extract.

PROFESSOR OTTO¹.

In checking the original report of Dr. Blumenthal, I discovered that, on June 30th, the serological diagnoses reported by him were erroneous in regard to a certain number of sera, because the reading

¹ See also Annex 2.

on that day differed from his usual procedure. The results of the following tests should therefore read:

Serum 584	+	instead of	±
„ 586	+	„	±
„ 587	+	„	±
„ 589	+	„	±
„ 594	+	„	±
„ 595	+	„	±
„ 598	±	„	—
„ 600	±	„	—
„ 604	±	„	—
„ 635	+	„	±

PROFESSOR SACHS.

Professor Sachs desires to put forward the following considerations in connection with the critical estimate of the value of the material collected at Copenhagen.

The essential factors in the utility of any process of serological diagnosis for syphilis are sensitivity and specificity. In an estimate of the diagnostic value of a test, specificity must carry the greater weight. With the material in question, it is difficult to judge of the specificity, because all the tests under consideration — even those practised separately by individual methods — which yielded, by anamnesis, indications of latent syphilis, were taken as characteristic for syphilis. It is impossible to decide *a priori* whether in such cases the reactions are specific or non-specific. Accordingly, it must be remembered that the results in reference to the specificity factor are somewhat less satisfactory than would appear from the recapitulatory tables. In addition, a complete estimate of the value of the material must take account of the ± reactions, because experience shows that on repetition ± reactions sometimes give a +, and, conversely, + reactions may give a ±. For this reason, ± reactions in control cases should always be regarded as borderline reactions, which are equally capable of turning out as non-specific reactions in certain circumstances. The actual factor of sensitivity and specificity would then be found by adding the + and ± reactions together.

PROFESSOR A. VERNES.

The Serological Conference held by the League of Nations at Copenhagen in 1928 with the object of improving the general conditions necessary for the campaign against syphilis offered an excellent opportunity for representatives of sixteen different countries to meet and compare, by conducting experiments on about a thousand sera, the methods which they regarded as giving the best results. All this work, for which the preparations were carried out in a manner that does credit to the ability of its organisers, was conducted in an atmosphere of extreme courtesy and with a universal anxiety to achieve some progress.

In this spirit, the Institut prophylactique was obliged, in order to assure its co-operation in the Conference's work, to abandon

all its usual principles and all its methods for the time being; and we feel bound to give an explanation here.

1. In the days when it was almost impossible to detect syphilis except by skin or mucous-membrane lesions, these lesions were described as primary, secondary or tertiary according to their appearance.

In that distant period, it was not suspected that syphilis might take a different course.

We now know that manifestations of the secondary type (mucous patches) may be observed in the most advanced stages of the disease, and conversely manifestations of the tertiary (gummata) may appear even in the first stages.

Further, we know that the immense majority of syphilitic cases present no apparent symptoms, but that the expression "latent syphilis" itself merely conceals localisations which could only be established by very careful medical exploration: mediastinum, kidneys, liver, heart, ductless glands, Erb's paraplegia, etc., and, above all, that syphilitic meningitis which produces not the slightest disturbance of the reflexes until it has attacked the nerve centres and ducts enveloped by the meninges and which, if not promptly checked, ends in tabes and various forms of encephalitis.

All this shows the necessity for close contact between serology and clinical practice, so that serology, based primarily on the firm foundation of careful medical observation, may in due course furnish the doctor with accurate results.

2. It was through sustained clinical observation that we were led nearly twenty years ago to the fundamental conclusion that serology would be condemned to remain a rudimentary science if it could only produce + or - results, while in clinical practice and in all the other laboratory sciences *fluctuations* are observed and noted.

By making a protracted graphical study of these fluctuations in the blood and cerebro-spinal fluid of the same subjects, who were kept under constant clinical observation, we succeeded, by repeated improvements of our technique, in recording these fluctuations on a scale ranging from 0 to 150 and upwards, all personal influence on the part of the operator being eliminated.

At the Conference, however, in order that our results might be comparable with the others, we were asked to express them by the symbols +, \pm and -, which we thought we had buried for ever when we established our first scale in 1910.

After some consideration, we agreed to make this temporary concession, as we saw no serious objection to it.

For one thing, no one smiles nowadays, as they did when I was beginning to demonstrate that "accurate serology throws light on the treatment of syphilis", and when only perhaps two foreign writers, Boas and Blaschko, shared my view. Also it was obviously useful that those who think only in terms of -, \pm , +, or even ++ and further multiples of +, should be shown that a method of measurement mechanically regulated to throw light upon the treatment throws light also upon the diagnosis.

It must be also remembered that many sera were not in a sufficiently preserved state for our reaction.

Alteration of the serum due to age can produce a slight flocculation in the non-syphilitic sera, and can sensibly diminish the flocculation in cases of syphilis. A number of the results shown in the

tables must be set aside for this reason¹, because it is apparent that the comparisons must bear on the modifications of the serum resulting from syphilis, and not on those which result from putrefaction.

Our method of measurement takes particularly into account the control of treatment, which gives it, in consequence, a fineness of diagnosis, on condition, I must repeat, that the sera are in a state of preservation such as we have in Paris. They should be used fresh, or have not been in transit more than three or four days, and all the usual precautions should, of course, be taken as regards temperature and aseptis.

Cerebro-spinal fluids should, however, be always examined on the same day as they are taken in the same way as for the leucocytosis test.

OBSERVATIONS UPON GUINEA-PIG COMPLEMENT BY DR. E. J. WYLER

For the Bordet-Wassermann tests carried out at this Conference by the Harrison method, complement was prepared as at home, the serum being taken off the clot four hours after the animal has been killed. The titres of the complements obtained in Copenhagen have for the most part been low as compared with those generally obtained at home, using the same hæmolytic amboceptor and the same strength of corpuscule suspension as ascertained by hæmoglobin measurement (Bigger's method). The titre of complement at home rarely falls below 1/60. The titres of complement (three guinea-pigs pooled in equal amount) obtained at this Conference were as follows:

May 21st: About 1/40.

May 22nd: do.

May 23rd: 1/30. The same result was obtained:

- (1) With two further samples of blood corpuscles;
- (2) With a different amboceptor;
- (3) With double the usual sensitisation.

May 24th: 1/55.

May 25th: 1/30. When the three component complements were separately titrated, two of them gave no lysis at 1/30, while the remaining serum gave a higher titre than 1/40 (no higher dilution than 1/40 was tried).

¹ Especially for the most obviously bad sera (chylous sera, granular sera, whether accompanied or not by the phenomenon of "silky waves" (*ondes soyeuses*), which we endeavoured to improve so far as possible by complications of technique (prolonged centrifugation, more-frequent test reading in the course of the experiment). Some of the numbers are given below:

3	114	190	254	329	393	472	599	729
4	121	191	255	332	394	476	600	737
6	123	192	256	333	401	479	601	738
9	128	193	264	334	402	485	602	751
15	138	194	265	335	403	490	623	807
24	139	195	266	336	404	491	625	813
32	140	196	274	339	412	511	632	839
49	143	199	277	346	413	513	634	855
53	146	203	280	347	415	518	639	876
63	150	215	281	349	417	520	641	892
64	151	216	282	353	430	543	648	893
65	153	218	283	354	432	548	654	897
73	171	220	284	357	438	556	667	899
78	173	226	285	361	439	578	668	902
79	174	228	286	362	446	582	677	903
84	179	229	294	373	450	583	683	924
96	183	233	295	376	453	584	688	925
104	184	244	296	377	455	588	693	929
108	187	245	306	378	459	595	694	937
109	188	248	317	386	461	596	717	941
113	189	253	327	392	470	597		

May 26th: 1/45. The three component complements, when separately titrated, gave about the same titre.

May 29th: 1/45. Ditto.

May 30th: 1/75. This complement was composed of two sera pooled in equal amounts. A third serum gave a slightly lower titre. The complement used on this day by the other delegates (consisting of the serum of numerous guinea-pigs) was also titrated by me and gave 1/40.

Three further complements of single guinea-pigs gave respectively $>1/60$, $>1/60$ and $1/40$.

May 31st: 1/70. This was the same complement as that used on the previous day, which had been kept frozen overnight. The mixed complement used by the other delegates gave 1/40.

June 1st: 1/70. This was the same complement as that used on the previous day (and also on May 30th) which had been kept frozen overnight.

In the technique under consideration, the complement is titrated in presence of excess of amboceptor. Low complement titres tend to decrease the sensitiveness of the test. Thus a serum tested at the conclusion of the Conference Tests with three different complements giving titres of 1/40, 1/70 and 1/70 respectively (the other ingredients of the test being, of course, the same) gave + with the weak complement and ++ with the other two. With seven other weakly reacting sera there was generally a tendency for a slightly increased degree of lysis with the weaker complement.

These low complement titres obtained at Copenhagen are of interest. Four of my guinea-pigs were despatched by aeroplane from London, two of these were used in the comparative tests indicated in the preceding paragraph. These were the two animals which yielded a titre of 1/70.¹ My animals are fed exclusively on oats and cabbage; the Institute guinea-pigs on barley, oats, beetroot and hay. Is it possible that this is connected with the differences of complement titre? I am not aware of any work in this connection except that of Griffith and Scott (Ministry of Health, London), who found that, when guinea-pigs are fed exclusively on green food, they get very thin, but their complement titre is not affected.

Certainly the animals used at the Serum Institute are often small. For my own work, I make it a rule to use only well-grown male animals (fed as stated) weighing usually about 700 grams. They are always weighed before use and if they have lost weight are rejected. They are never used for inoculation purposes.

¹ The sera of the remaining two guinea-pigs were titrated a few days later and gave respectively 1/80 and 1/90.

Appendix

NOTE ON THE PREPARATORY WORK CARRIED OUT AT THE
PROPHYLACTIC INSTITUTE (PARIS) FOR THE COPENHAGEN
CONFERENCE, PRESENTED BY DR. VERNES, DURING
THE CONFERENCE.

Gentlemen,

I wish to bring to your notice the work that the Paris Prophylactic Institute has undertaken for the Copenhagen Conference. We have investigated certain tests for flocculation, comparing them with our own reagent, which, as you know, has remained absolutely unchanged since March 1917. The method we now follow, after successive improvements in our optical material, has not been changed since 1921. The work involved in the studies for this Conference has been considerable.

We received the material for the Sachs reaction on January 12th and have carried out 2,320 tests up to date.

The material for the Meinicke reaction was received on January 12th and 2,320 tests were made.

We received the material for the Sigma reaction, as modified by Dr. Madsen, on March 14th, and have carried out 580 tests.

The material for the Kahn reaction reached us on March 24th and has been employed for 640 tests.

Thus, including our current work, we have carried out 25,205 sero-reactions in our laboratory during this period, in addition to 4,796 serological tests for tuberculosis.

At the request of the President of the Health Committee, Dr. Madsen, and the Director of the Health Section, Dr. Rajchman, 128 specimens of cerebro-spinal fluid were sent by air mail from Paris to Copenhagen.

These tests were carried out in the laboratories of the Paris Prophylactic Institute (36, rue d'Assas) with, I assure you, just as much meticulous care as if we had performed them ourselves. The interests of the patient have been paramount.

So, in addition to the notation which had been suggested to us, we have added the photometric notation for the two reactions which lend themselves to it, namely, the Sachs and Meinicke tests.

When measured with the photometer (this is employed nowadays for all kinds of ultra-fine measurements in serological work: urea, cholesterolin, calcium, phosphates, magnesium, glucose, uric acid, albumin, etc.), both these tests, particularly the Sachs reaction, have much in common with our own, in that they tend themselves to a graphic representation based on photometric indications. With so little time before us, we could not do more than begin a comparative study of this nature; but, now that we are provided with the necessary organisation, we propose to continue it during the coming months.

We can only submit the beginnings of charts, chiefly of syphilitic cases put under treatment immediately on arrival, with the descending curve following treatment.

Another part of our documentation concerns cases which, judging by our experience — our observations extend over an uninterrupted period of seventeen years — can be regarded as cured.

The following points must be borne in mind: the great difficulty has been to establish a definite line of demarcation between syphilitic and normal sera, since syphilitic flocculability is simply an exaggeration of normal flocculability, just as fever is merely an exaggeration of the normal temperature in any individual. Further, normal subacute flocculability remains invariable in any given person (producing a horizontal line in the chart), whereas the distinctive feature of superadded syphilitic flocculability is that it may be characterised by very marked fluctuations. Again, and this is most important, in a person in whom the cerebro-spinal fluid is normal, but in whom syphilis has not been completely eradicated, an upward curve invariably occurs within eight months of the last injection of arseno-benzene, whereas, once the chart line has remained low for eight consecutive months after such injection (arsenical signpost — *jalon arsenical*), with normal cerebro-spinal fluid on the expiry of that period, no subsequent rise in the curve has ever been observed during our seventeen years' experience.

*

* * *

The ideal test would be in the nature of an alarm signal recording the entry of the first microbe and ceasing to register on the death of the last survivor.

As this is not feasible, we have to consider what is the best test for practical purposes. This test, in our view, should show a reaction directly after the introduction of the treponema, should be absolutely specific for syphilis, and should give the same objective result to any operator at any time.

We now come to the famous question of sensitivity.

The mere fact of a reaction showing an upward tendency more promptly or to a greater degree in a given subject is insufficient to prove that it is more sensitive; it must also drop, under the influence of treatment, below its starting-point (otherwise the starting-point does not represent an increase in flocculability). In view of what we already know concerning the variability of syphilitic flocculability as compared with the invariability of any normal case, the position of zero, as determined by nature, is placed at the lowest point to which the curve can be made to drop as the result of treatment, and only a series of observations can determine this basic line of invariability.

As regards our own experience, we have at our disposal the patient and his humours and we never take any step in serology that is not supported by clinical observation or any step in medical practice that is not borne out by the results of serological research. After many years of experiment, we are now able to lay down the conditions necessary for a proper control of the effects of various drugs. In virtue of this method, we now propound the principle that true prophylaxis of syphilis consists in rendering the patient innocuous, not for a certain period, but for life — in his own personal interest and as the only means of successfully combating the terrible scourge of hereditary syphilis. In this way we have brought about the technical organisation which we are still endeavouring to perfect for our country with the aid of Parliament, the State, the Paris Hotel de Ville and numerous organisations.

Prolonged observation appears to us essential if we are to make a really useful contribution towards a comparative study, as we have been requested to do. We ask nothing better than to preserve this study as long as may be necessary along the lines which we have organised in Paris.

All who have devoted themselves during the last twenty years to exhaustive researches on the serology of syphilis have come to the same conclusion, namely, the importance of a minute analysis of the phenomena of flocculation. The sanction of this Conference will thus mark an immense progress.

If the Health Organisation of the League of Nations would do all in its power to promote the practical application of this principle in the supervision of syphilis treatment, it will have done much to assist the methodical organisation of the various means designed to eradicate this disease all over the world.

I may add, since we propose to pursue our comparative studies during the coming months in a spirit of perfect objectivity and impartiality, that we shall be only too glad to receive suggestions and information from the different authors in regard to their technique.

Comparative studies were carried out at our Institute between January 12th and May 26th on upwards of eight hundred patients. We have only been able to submit a limited number of charts (two hundred) owing to the clerical work involved. Should you be willing for the next Conference to be held at the Paris Prophylactic Institute, I can assure you, on behalf of the Committee which I have the honour to represent here, that its laboratories and services would be placed entirely at your disposal and that everything possible would be done to facilitate your work. You will find all the sera and specimens of cerebro-spinal fluid you can possibly need, taken on the actual day, from patients under observation or undergoing treatment whose clinical history is recorded in our archives.

You may rest assured, gentlemen, that your visit would meet with a most sympathetic welcome from the French authorities, and, in order to convey to you the warm welcome which would be extended to you, I am authorised to inform you that the President of the Republic will be happy to receive you and to express in person his interest in your activities.

Annex 2.

DESCRIPTION OF THE VARIOUS METHODS USED¹

METHOD USED BY PROFESSOR D. DE BLASI.

1. Method:

The classic Bordet-Wassermann method, except that on each occasion the hæmolytic system is first adjusted according to the method described below. A suspension of red blood corpuscles is used, the concentration of which is variable, but which always contains the same "hæmolysable mass" determined by reference to a colorimetric standard. The "*vis complementaris*" of the guinea-pig serum is then titrated.

2. Principles:

If the discrepancies between the results of the serodiagnostics methods based on complement fixation are to be reduced, results must above all be capable of comparison with each other. The comparability of the results of the B.-W. reaction obtained with the same sera by several operators is none other than the comparability of the antigens used. In order that a comparison between several antigens may show which of them is the most sensitive and at the same time the most specific, *the antigens must be titrated by all the operators with the same doses of complement, of amboceptor and of "hæmolysable mass"*. But the complement must be titrated before the antigen and the two other elements must therefore be constant. One amboceptor, once it has been titrated, is used throughout in a constant dose until the supply is exhausted. But the quantity of corpuscles, or, more precisely, of the "hæmolysable mass" is not always equally constant. In fact, 5% suspensions prepared in different laboratories (sometimes even in the same laboratory, but at different times) do not always contain the same "hæmolysable mass". This explains why the results of the titration of the "*vis complementaris*" of the same guinea-pig serum may work out differently for different operators, which necessarily involves a difference between the results of the titration of the same antigen. In view of these considerations, the method was tried, in 1918, of rendering

¹ The following descriptions have been supplied by the participants themselves, any appreciation of the single method must therefore be regarded as the personal opinion of the author concerned.

the “*hæmolysable mass*” constant with the help of a constant colorimetric standard of reference. (See the Memorandum “Sulla determinazione del sistema emolitico nella reazione di Wassermann”, DE BLASI, *Bollettino della R. Accademia di Medicina di Roma*, A. XLV, 1918-1919.)

3. *The advantages of the preliminary determination of the hæmolytic system are as follows:*

(a) By consistently using the same quantity of amboceptor and the same “*hæmolysable mass*”, the “*vis complementaris*” of guinea-pig serum is made really constant in all experiments carried out at any time in the same laboratory; (b) further, an almost perfect comparability between the hæmolytic systems used in laboratories situated at a great distance from one another could be assured if the same colorimetric standard of reference were adopted by agreement everywhere; (c) finally, once the composition of the hæmolytic system was made constant *always and everywhere*, and once the antigen was dosed with such a system, a more satisfactory comparability of the B.-W. results would be obtained, i.e., a more reliable comparative estimate of the quality of the various antigens, independently of the time and place where the reactions are carried out.

4. *Temperature:*

The sera are inactivated at 53° C. for half an hour in a water-bath. The mixtures “serum + antigen + complement” are kept for an hour at 37° C. in a water-bath. After adding the mixture “amboceptor + corpuscles”, the tubes are replaced in the water-bath, from which they are removed as soon as the controls permit.

5. *Reading results:*

Two readings are taken: the first after the removal of the tubes from the thermostat; the second half an hour later. The results according to the second reading are taken as final. If the results of the first reading are ++ or + and those of the second ± or —, the reaction is always repeated with a fresh blood sample.

6. *Number of tubes required for the control of one dose of serum:*

One tube containing the same dose of serum as the reaction tubes.

7. *Pipettes required for each test:*

- (a) For each serum, a 1 or 0.5 c.c. pipette, graduated either in 100ths or 200ths;
- (b) Three pipettes for the saline: one 2 c.c. in 100ths, one 5 c.c. in 10ths, one 10 c.c. in 10ths;
- (c) Two pipettes for the complement: one 2 c.c. in 100ths (for the titration), one 5 c.c. in 10ths (for the distribution);
- (d) Two pipettes for the antigen: one 2 c.c. in 100ths, one 5 c.c. in 10ths;
- (e) One 1 c.c. pipette in 200ths for the amboceptor;
- (f) Two pipettes for the corpuscles: one 2 c.c. in 100ths (for the determination of the “*hæmolysable mass*”), one 5 c.c. in 10ths (for the distribution of the mixture “amboceptor + corpuscles”).

8. *Other apparatus required:*

Small graduated glass tubes, one of which contains a formol-acetic solution of acid fuchsin, the colour of which matches that of the lakage of a dense mass of washed corpuscles, diluted in the proportion of 1:200.

Adjustment of the Hæmolytic System.

1. *Determination of the quantity of blood-corpuscle suspension.* — The first stage is a colorimetric process which consists in laking one volume of corpuscle suspension in 99 volumes of distilled water (1:100) and in comparing the colour of the solution with that of a standard colour solution. At first, Professor de Blasi used Gower's hæmoglobinometer, but subsequently came to prefer a formol-acetic solution of acid fuchsin and picric acid, the chromatic intensity of which corresponds to a thick red-cell suspension laked 1:200. The composition of the liquid used as a standard of reference is that given by Vernes in the *Comptes rendus de l'Académie des Sciences*, 1918, No. 14. The colorimetric procedure is described in the paper on "The Adjustment of the Hæmolytic System in the Wassermann Reaction" (*Bollettino della Reale Accademia di Medicina di Roma*, A. XLV, 1918-19).

2. *Dosing of complement.* — The second preliminary stage consists in the determination of the minimum hæmolitic doses of guinea-pig serum, using twelve different concentrations, ranging by regular degrees from 2:1000 to 13:1000. By multiplying the minimum concentration which produces hæmolysis by the empirical constant of 2.5, the concentration of complementary serum is obtained which is to be used both for the dosing of antigens and for the B.-W. test. This second stage is described in the paper on the "Method of ensuring Constancy of Effect with the Hæmolytic System" (*Archivio di scienze biologiche*, Vol. V., Nos. 1-2, 1923).

The first of the two preliminary stages mentioned ensures the use of a constant "hæmolysable mass" at all times by one and the same worker; and could do so in the case of several workers if they came to an agreement. The second stage makes it possible always to use the same quantity of complement which may be present in varying amount in the sera of different guinea-pigs. Taken together, the two processes ensure comparability of results with the hæmolytic systems used on various days in one and the same laboratory; and they could do this with the hæmolytic systems used in various laboratories, provided the colorimetric liquid which serves as a standard of reference and the titration of the complement were the same.

METHOD EMPLOYED BY DR. E. DEBAINS.

1. *Method employed:*

The Calmette and Massol method applied to *unheated sera* by super-activation procedure.

2. *Principles:*

The original method of Calmette and Massol (heated sera). Use of a fixed dose of antigen; increasing doses of complement — E. Debain's modification; use of human complement (unheated sera); superactivation by increasing doses of guinea-pig complement.

3. *Advantages:*

(a) A greater sensitiveness:

Alteration of the sera by heating at 55° C. is avoided;

The component parts of the hæmolytic system can be measured accurately; for each tube containing antigen there is a control tube which is strictly comparable.

(b) The strength of the reaction can be measured by this method.

This sensitiveness is particularly valuable in the detection of unsuspected latent syphilis—pseudo-syphilis.

The method, however, requires the use of fresh sera; the samples must be taken twenty-four or forty-eight hours before the test.

4. *Temperature:*

37° C. (1½ hours).

5. *Reading of results:*

After half-an-hour. (The racks are inspected; if the hæmolytic activity of a serum is low, the reading must be postponed till hæmolysis is complete in the control tubes.)

6. *Tubes necessary for each serum:*

Nine tubes are necessary for each serum: four control tubes and five containing antigen.

7. *Pipettes required:*

One pipette per serum.

8. No other apparatus is necessary.

9. *Other details concerning the Calmette and Massol method applied to unheated sera; superactivation procedure:*

Preparation of the Antigen (horse-heart extract). — The heart, having first been pounded, is dried in a vacuum at 30° to 35° C. in an ordinary desiccator which can work large quantities; the operation only requires a few hours. The dry matter is pulverised and sifted by machinery. The powder thus obtained is homogeneous and very fine. The fatty matter is removed by successive extractions with

acetone at room temperature. To make sure that all the fatty matter has been removed, excess of water is added to the decanted acetone ; the mixture should remain clear. The powder, freed from fat, is dried in the incubator at 37° and placed for 15 days in absolute alcohol, in the proportion of 20 gr. of powder to 100 gr. of alcohol. The liquid is decanted and filtered. For the test the alcoholic extract is measured with a pipette into a flat-bottomed cylindrical vessel, and physiological saline is added in the proportion of 25 c.c. to 1 c.c. of extract. The saline is added in two stages: first 5 c.c. of saline for each c.c. of extract are added drop by drop, imparting at the same time to the cylindrical vessel which is held in the left hand a circular motion in order that the saline may mix thoroughly with the extract. The remainder of the saline is then poured down the side of the vessel and the whole gently mixed.

Reaction. — Nine tubes are used for each serum: four control tubes containing 0.8 c.c. of saline, five reaction tubes containing 0.8 c.c. of colloidal suspension (antigen). Doses of complement diluted 1:25 are then delivered into the tubes according to the table given below:

	I	II	III	IV	V
Control tubes	0	0.1	0.2	0.3	—
Reaction tubes	0.1	0.2	0.3	0.4	0.5

Although the antigen has no anti-complementary action, each reaction tube is given one more dose of complement than the corresponding control tube.

Increasing doses of complement are added to all the tubes in fixed quantities of 0.8 c.c. For this purpose, five dilutions of complement in an increasing ratio of concentration are prepared, *e.g.*, for 80 sera the following solutions must be prepared:

	Saline	Guinea-pig serum
1.	160 c.c.	1 c.c.
2.	160 c.c.	2 c.c.
3.	160 c.c.	3 c.c.
4.	80 c.c.	2 c.c.
5.	80 c.c.	2.5 c.c.

After these have been in the incubator for one and a-half hours, 0.8 c.c. of a suspension of sensitised red sheep corpuscles are added.

The total volume in each tube is 2.5 c.c. (0.8 c.c. of saline or antigen plus 0.8 c.c. of complement, plus 0.1 c.c. serum, plus 0.8 c.c. sensitised red corpuscles.)

The quantity of corpuscles contained in 0.8 c.c. should be such that hæmolysis is not complete in the first control tube. This quantity has been calculated by taking into account the average hæmolytic power of human sera.

The corpuscles, well washed in saline, are centrifuged for ten minutes. At the end of that time, the volume of the deposit has

reached its maximum. The volume of this deposit multiplied by 2.8 indicates the quantity of saline to be added.

Example: Volume of the deposit = 50 c.c.

Total volume of the emulsion = $50 \times 2.8 = 140$ c.c.

Quantity of saline to be added = 90 c.c.

METHOD USED BY DR. L. W. HARRISON
AND DR. E. J. WYLER.

1. *Method used:*

No. 1 method, Medical Research Council, Special Report Series No. 14, as modified by Dr. E. J. Wyler.

2. *Principle:*

Complement fixation: the essentials of the technique are set out below.

3. *Advantages:*

It would appear that the principal advantage of this method consists in a high degree of specificity without undue loss of sensitiveness.

4 and 5. *Temperature and reading of results.*

Fixation takes place for 30 minutes at room temperature followed by 30 minutes in the water-bath at 37° C. The sensitised sheep cells are then added and the racks replaced in the water-bath at 37° C. The results are read as soon as the negative control serum shows complete lysis.

6. *Tubes used for each serum:*

Four.

7. *Number of pipettes used:*

In Donald's "dropping" technique which was employed at the Copenhagen Conference, graduated pipettes are not needed in the test proper. One dropping pipette is used for the sera and is washed out with saline between each specimen; one dropper is used for saline, complement dilutions, and sensitised cells; and one other dropper is used for antigen suspension.

8. *Apparatus used:*

A burette to which Donald's droppers are attached as described in Medical Research Council, Special Report Series No. 14, pages 22-27.

9. *The following are the essentials of the method:*

Inactivation of patient's serum. — Thirty minutes at 55° C.

Complement. — Guinea-pig's serum titrated as follows:

This is done on every test-day, the volume of fluid in each tube being the same as in the test proper. Two rows of tubes containing graded strengths of complement dilution are set up as follows:

- Row 1 contains 2 volumes saline;
 - 1 volume complement dilution;
 - 1 volume sensitised cells.
- Row 2 contains 1 volume saline;
 - 1 volume complement dilution;
 - 1 volume antigen suspension.

Row 1 is incubated for thirty minutes at 37° C. and the titre of the complement is then read.

Row 2 is incubated for thirty minutes at room temperature and then for thirty minutes in the water-bath at 37° C. At the end of this period, a volume of sensitised cells is added to each tube and the rack replaced in the water-bath at 37° C. for thirty minutes. Provided that the result of the final incubation of row 2 shows that the titre as ascertained in row 1 has not been reduced through the presence of antigen by more than one dose the reading obtained in row 1 is accepted.

Antigen. — Alcoholic human heart extract (three parts); 1% alcoholic solution cholesterol (two parts). These are mixed on the day of the test and the mixture diluted 1 in 15 with normal saline by pouring the saline quickly upon it.

The heart extract is prepared as follows: the fat having been cut away, the muscular portion of the left ventricle is minced and ground for one minute with absolute alcohol (1 grm. of heart muscle to 9 c.c. absolute alcohol) in a mortar with powdered glass. The mixture is placed in the incubator at 37° C. for twenty-four hours, being shaken at intervals. At the end of twenty-four hours it is filtered, placed in the ice-chest overnight and again filtered. The extract remains unchanged at room temperature for many months.

Hæmolytic system. — A standardised suspension of sheep's red blood cells sensitised with six doses of hæmolytic immune body.

Test proper. — Four tubes are required for each serum.

Each tube contains the same quantity of fluid as follows:

- | | | |
|---------------------------|---|--|
| Tube 1
(serum control) | } | 1 volume saline;
1 volume patient's serum diluted 1 in 5
with saline;
1 volume complement dilution in saline,
containing three minimal hæmolytic
doses. |
| Tube 2
(serum control) | } | Same as tube 1, but the volume of comple-
ment dilution in saline contains two
minimal hæmolytic doses. |

Tube 3	{	1 volume patient's serum diluted 1 in 5 with saline;
		1 volume complement dilution in saline containing five minimal hæmolytic doses;
		1 volume antigen suspension.
Tube 4	{	Same as tube three, but the volume of complement dilution in saline contains three minimal hæmolytic doses.

The tubes are incubated first at room temperature for thirty minutes and then in the water-bath at 37° C. for thirty minutes, after which one volume of sensitised cells is added to each and the racks replaced in the water-bath at 37° C. The reading is taken in thirty minutes or earlier if the negative control serum shows complete lysis.

Cerebro-spinal fluid. — This is tested in the same way as serum (one volume of C.S.F. diluted 1 in 5), except that a further three rows of tubes are put up containing two, five and ten times this amount.

METHOD EMPLOYED BY DR. E. JACOBSTHAL.

Method used:

Synoptic cold method (Jacobsthal).

Principle:

In order to gauge the strength of the reaction, simultaneous tests of varying sensitiveness are made with the same serum, namely:

- (1) "The Jacobsthal cold method" with cholesterinised alcoholic heart extract (human, or perhaps ox, heart extract);
- (2) The "warm method" with the same extract;
- (3) The "cold method" with non-cholesterinised alcoholic heart extract;
- (4) The "warm method" with the same extract.

As a rule, one dose of each extract is employed and not graduated doses. The tests are made with "quarter doses" (total volume 1.25 c.c.). Complement diluted 1 in 10; amboceptor titrated on every test-day: 2 ½ doses. Patient's serum inactivated and diluted 1 in 10.

In the *cold method*, the serum and antigen mixture combined with complement is shaken, immersed in a salt and ice-water mixture and placed in the ice-box for one hour. The hæmolytic system having been meanwhile prepared, the sensitised cells are added at the expiration of the hour and the tubes put in the incubator at 37° C. for a further hour. In the *warm method*, the mixture of antigen and serum is incubated in the usual manner at 37° C. for an hour, during which the hæmolytic system is prepared. Incubation as above.

With very rare exceptions, the cold reactions are far more sensitive than the corresponding warm reactions; again, the reaction is more sensitive with cholesterinised than with non-cholesterinised extract. For this reason, No. 2 of the above-mentioned reactions is sometimes stronger and sometimes weaker than No. 3.

The cerebro-spinal fluid is never tested by Jacobsthal's cold method and never with cholesterinised extracts, because unspecific results may be obtained, especially in isolated cases of tuberculosis.

But in every instance a graded test is made according to Hauptmann's method in the following doses: 0.05, 0.1, 0.5 and 1.0.

METHOD EMPLOYED BY PROFESSOR R. OTTO
AND DR. BLUMENTHAL.

1. *Method used:*

The original Wassermann Reaction.

2. *Principles:*

Complement fixation. The results obtained with syphilitic liver extract adjusted to give sensitive reactions are primarily taken into account.

3. *Advantages of the method:*

Detection of a wide range of cases, especially *Lues latens* and *Lues congenita*.¹

4. *Temperature:*

In the incubator at 37° C.

5. *Reading results:*

After fixation for one hour in the incubator, the hæmolytic system is added. Further incubation until the controls are lysed. This requires on an average another half-hour.

6. *Number of tubes required for a test:*

Four.

7. *Number of pipettes required:*

In addition to 1 pipette each for saline, blood, complement, amboceptor, and extract, one pipette is required for each serum.

8. *Other apparatus required:*

A water-bath at 56° C. and an incubator at 37° C.

9. *Other details of the technique:*

Government-tested extracts are used as antigens, *i.e.*, the syphilitic liver extract adjusted to give sensitive reactions with an

¹ In the Serological Department of the Robert Koch Institute, a parallel flocculation reaction is carried out (*cf.* R. Otto, *Klin. Woch.* 1925, page 1312).

admixture of guinea-pig's heart extract, employed with $\frac{1}{10}$ and $\frac{1}{15}$ complement (cf. G. BLUMENTHAL, *Medizinische Klinik*, 1926, No. 51), and a less strongly cholesterinised extract of ox-heart.

The dose of amboceptor to be used is determined in the preliminary test by two readings (in twenty minutes and again in an hour), and comparison of the values thus obtained. (Cf. G. BLUMENTHAL, *Berliner Klinische Wochenschrift*, 1921, page 1067.)

METHOD EMPLOYED BY DR. D. PAVLOVITCH.

1. The method of serum diagnosis of syphilis practised at Copenhagen is based on the complement fixation principle of *Bordet-Wassermann*.

2. Heated sera are used, with three different antigens and a fixed dose of complement. The antigens are as follows: (1) Bordet's antigen, (2) two alcoholic extracts of ox-heart *without cholesterin*. The hæmolysin is titrated daily with guinea-pig complement taken the same day. The lay-out of the test may be seen from the following table:

Tubes	I	II	III	IV
Heated serum	0.10	0.10	0.10	0.20
Antigen I	0.25	—	—	—
Antigen II	—	0.25	—	—
Antigen III	—	—	0.25	—
Complement 1/10	0.25	0.25	0.25	0.25
NaCl Solution	0.20	0.20	0.20	0.40

One and a-half hours in the water-bath at 37° C.

Hæmolytic system. 0.5 c.c. of a 5% suspension of sheep red corpuscles is added to each tube.

Half-an-hour in the water-bath at 37° C.

The antigens are diluted by means of the *Bigger* apparatus used for the Sigma reaction.

The amount of hæmolysin necessary for the reaction is determined in two stages: first, the minimum hæmolytic dose with the fixed quantity of complement is ascertained and then the complement is titrated with two, three (and four) doses *in the presence of antigen*, according to the following arrangement:

Tubes	1	2	3	4	5
2 to 4 Minimal Hæmolytic Doses	0.25	0.25	0.25	0.25	0.25
Complement 1/10	0.10	0.15	0.20	0.25	0.30
Antigen	0.25	0.25	0.25	0.25	0.25

Half-an-hour at 37° C. Then 0.25 c.c. of 5% suspension of red corpuscles to each tube.

For the test, the dose is taken which, with 0.20 c.c. of the complement, produces complete hæmolysis:

3. *The advantages of this method are:*

- (a) Our non-cholesterinised antigens have very rarely given non-specific results. The reaction is perhaps rather less sensitive than the others, but we prefer to maintain specificity even at the expense of sensitiveness. The antigens are always diluted in the same manner.
- (b) The method saves time, a great advantage in laboratories in which tests are made on a large scale, for all the titrations are carried on after the incubation of test proper at 37° C. has commenced.
4. The reaction is carried out in the water-bath at 37° C.
5. The reading is taken immediately on removal from the water-bath, *i.e.*, two hours are required to obtain the result.
6. Four tubes are used for each serum.
7. One worker uses about five pipettes of 1 c.c. divided into $\frac{1}{100}$ ths for the distribution of the sera. After use, a pipette is washed with saline solution, and is left to drain on filter paper while the others are in use. In addition, one 5 c.c. pipette is required for the complement, one for the antigen, and one 10 c.c. pipette for the hæmolytic system.
8. The other apparatus required consists of a 37° C. and a 56° C. water-bath and a dropper.

METHOD EMPLOYED BY DR. S. SIERAKOWSKI.

1. *Method used:*

McIntosh and Fildes' method for the Bordet-Wassermann reaction.

2. *Principles:*

- (a) Preliminary titration of the complement on each test-day;
- (b) Dose of hæmolytic serum fixed in advance;
- (c) Fixed dose of antigen.

3. *Advantages of the method:*

- (a) Titration of the complement as a preliminary;
- (b) Titration of the complement in the presence of antigen.

4. *Temperature:*

37° C.

5. *Reading result:*

The titration of the complement takes 45 minutes; the reaction itself 45 minutes.

6. *Number of test-tubes used for each serum:*

Using a single antigen: two tubes — one for the serum plus antigen and one for the control.

7. *Number of pipettes used for each experiment:*

One pipette for each serum, and a few extra for the reagents.

8. *Other apparatus required:*

Water-bath at 56° C.; water-bath at 37° C.

9. *Other details of the technique employed by Dr. Sierakowski:*

Use of two antigens:

(1) Alcoholic extract of ox-heart + cholesterin 1 per cent.

Antigen diluted 1 in 15 (0.6 heart extract + 0.4 cholesterin + 14 saline).

(2) Bordet-Ruelens antigen (evaporated). Diluted 1 in 40.

Fixation in the water-bath at 37° C. for 30 minutes.

Dose of serum: 0.1.

TECHNIQUE EMPLOYED BY DR. H. BOAS.

The method employed by Dr. Boas is the new *Kahn* method (the rapid method) exactly as described by Kahn in his book "Serum Diagnosis by Precipitation".

OUTLINE OF TECHNIQUE OF KAHN TEST.

1. *Method used:*

The Kahn test.

2. *Principles of this method:*

The Kahn test is a precipitation method for differentiating syphilitic from non-syphilitic serum with the aid of a specially prepared cholesterinised alcoholic extract. The test has been developed in conformity with the following requirements for optimum precipitation:

- (a) Optimum concentration of antigenic lipoids in the antigen: Excessive or deficient concentration preventing precipitation. This quantitative relation between the concentration of an antigen and its sensitiveness permits the preparation of an antigen having a standard degree of sensitiveness by the variation of the lipid concentration.
- (b) Proper physical state of the antigen suspension: The suspension must contain a minimum amount of physiological salt solution and the lipid aggregates must be dispersible.
- (c) Correct quantitative relation between serum and antigen suspension, an excessive amount of suspension being inhibitory to precipitation.

- (d) Shaking — as a probable aid in hastening collision between the serum-antigen interacting particles (optimum speed = 275 to 285 oscillations per minute).
- (e) Total dilution of the suspension — serum mixture should be a minimum for a given amount of mixture. Increasing the volume of the mixture with physiological salt solution (or with water) tends to reduce precipitation.

3. *Advantages of this method:*

Technical.

- (a) Practical steps evolved for bringing antigens prepared from different heart muscles to a standard degree of sensitiveness.
- (b) Stability of antigen.
- (c) The use of but three reagents — antigen, salt solution and serum.
- (d) Relative simplicity of technique, minimising thereby the sources of error.
- (e) Rapidity in obtaining results.
- (f) Availability throughout the world — in the tropics, field laboratories, etc.

Clinical.

- (a) High degree of specificity combined with sensitiveness.
- (b) Clear-cut reactions, minimum number of border-line reactions.
- (c) Several processes aside from the regular Kahn test as an additional aid to clinicians in their diagnosis and treatment of syphilis:
 - (i) Quantitative process with serum as an aid in studying the serological effect of therapy.
 - (ii) Qualitative process with spinal fluid corresponding to regular Kahn test with serum.
 - (iii) Quantitative process with spinal fluid as an aid in studying the serological effect of neuro-syphilitic therapy.
 - (iv) Presumptive processes with serum and spinal fluid, more sensitive than the regular processes owing to the employment of an antigen (sensitised) which is more potent than standard antigen.
 - (v) Special processes, including microprocesses with serum and spinal fluid, and process with chancre fluid.

Research.

The Kahn test especially lends itself to the application of research by physical and biological chemists as well as by serologists.

4. *Temperature at which the heating is carried out:*

- (a) The serum is heated for thirty minutes at 56° C.
- (b) The test is performed at room temperature.

5. *Reading of results:*

After three minutes' shaking.

6. *Test-tubes used for one serum:*

Three tubes.

7. *Pipettes used for one test:*

Three pipettes, two of which may be used for a series of tests.

8. *Other apparatus required:*

Centrifuge for separating serum from blood-clots.

Water-bath for heating serum at 56° C. for thirty minutes.

Test-tube rack.

In laboratories where large numbers of tests (thirty or more) are made daily, a shaking apparatus is of considerable help.

9. *Other details of the method:*

I. *Apparatus.*

1. Test-tubes for performing test are about 7.5 cm. in length and 1 cm. in diameter.

2. Vials (with straight wall and flat bottom) for preparing antigen suspension are about 5.5 cm. in length and 1.5 cm. in diameter.

3. Pipettes: 10 c.c. graduated to 0.1 c.c.; 1 c.c. graduated to 0.01 c.c. and 0.2 c.c. graduated to 0.001 c.c.

4. Test-tube rack: Made of sheet copper, 3 in. wide, 11½ in. long, 2¾ in. high. Consists of three shelves, upper and middle ones containing three rows of ten holes, each of approximately ½-in. diameter. The centre-row holes are offset half an inch.

5. Shaking apparatus may be of any construction that will hold the test-tube racks employed. The required speed is 275 oscillations per minute, with a stroke of 1½ inches.

6. Water-bath (56° C.); centrifuge and centrifuge tubes may be of any make that will be found convenient in the particular laboratory.

II. *Reagents.*

1. *Antigen.* For preparation and standardisation of antigen for Kahn test, see "Serum Diagnosis" and *Jour. Inf. Diseases*, Vol. 41, page 111, 1927. An antigen once standardised maintains its titre indefinitely. Antigen should be kept in the dark at room temperature. When subjected to cold, a precipitate may be thrown down, which can be re-dissolved upon warming in a water-bath. Only chemically clean and dry glass vessels should be used for storing antigen, and the cork stoppers should be covered with thin high-grade tinfoil.

2. *Serum.* The blood specimen should be centrifuged to remove clot and cells. The serum must be entirely free from cells or other particles. Previous to its use in the test, the serum is heated in a water-bath at 56° C. for thirty minutes. When serum that has been heated is kept overnight in the icebox, re-heating for ten minutes at 56° C. is necessary before using in the test.

3. *Physiologic salt solution.* A solution is prepared of 0.9% sodium chloride (chemically pure) in distilled water.

III. Routine (Diagnostic) Test with Serum.

This is a three-tube test. Each tube contains a different proportion of serum and antigen suspension according to the following outline:

Tube	1	2	3
Antigen suspension, c.c.	0.05	0.025	0.0125
Serum, c.c.	0.15	0.15	0.15

It is well to have everything arranged before mixing the antigen with salt solution for the test. Have racks set up, tubes numbered, sera heated and pipettes ready for reassuring antigen suspension and serum. For measuring the 0.05 c.c. quantities of antigen suspension, mark off these amounts on a 1 c.c. (graduated to 0.01 c.c.) pipette with a wax pencil. For measuring the 0.025 and 0.0125 c.c. quantities, use 0.2 c.c. (graduated to 0.001 c.c.) pipettes, on which these amounts are also indicated with a wax pencil.

Performance of Test.

1. *Preparation of Standard Antigen Suspension.* Mix antigen with salt solution according to required titre. Thus, if the titre is 1 c.c. antigen plus 1.1 c.c. normal saline, mix the antigen as follows: (a) Measure 1.1 c.c. saline into a standard antigen suspension vial; (b) measure 1 c.c. antigen into a similar vial; (c) pour the salt solution into the antigen, and as rapidly as possible (without waiting to drain the vial) pour the mixture back and forth six times to ensure thorough mixing; (d) allow the antigen suspension to stand for ten minutes before using. The suspension should not be used after thirty minutes' standing.

One may mix more than 1 c.c. of antigen with a proportionally larger amount of salt solution, but not less than 1 c.c. This amount when mixed with saline will be sufficient for about fifteen tests. Two c.c. of antigen mixed with saline will be sufficient for about thirty-five tests.

2. *Measuring antigen suspension.* After the antigen suspension has stood for ten minutes, shake it well and measure 0.05, 0.025, and 0.0125 c.c. amounts for each serum, delivering the suspension to the bottom of the tubes. When employing the standard rack which contains thirty tubes, measure 0.05 c.c. amounts in the tubes of the first row; 0.025 c.c. amounts in the tubes of the second row and 0.0125 c.c. amounts in the tubes of the third row.

3. *Measuring serum.* The serum should be added as soon as possible after the antigen suspension has been pipetted, to avoid undue evaporation from the suspension. When examining large numbers of sera, it is well for one worker to measure the antigen suspension and for another to follow with the sera. Add 0.15 c.c. serum to the 0.05, 0.025 and 0.0125 c.c. amounts of antigen suspension, and shake the rack of tubes vigorously for a few seconds to ensure thorough mixing of the ingredients. The rack can now be set aside

until the remaining tests are ready for the regular three-minute shaking period.

4. *Controls.* At least one positive and one negative serum control should be included with each series of tests. Every serum giving a positive reaction should be examined to establish that it is free from red cells or foreign particles which might be confused with a specific precipitate. Dilute 0.1 c.c. serum with 0.3 c.c. saline. Shake well and examine for particles. If particles are present, the serum should be recentrifuged and re-tested.

5. *Shaking.* During the three-minute shaking period, it is important not merely to agitate the rack of tubes, but to see to it that the fluids within the tubes are vigorously agitated. When shaken by hand, one may shake three one-minute periods with short rest periods. When a shaking machine is employed, its speed should be 275 oscillations per minute, with a stroke of 1.5 inches. Hand shaking should approximate this speed.

6. *Addition of saline.* After the tests have been shaken, add 1 c.c. saline to each tube of the first row of the rack (containing the 0.05 c.c. amounts of antigen suspension) and 0.5 c.c. saline to the remaining tubes. Shake sufficiently to mix ingredients

7. *Reading results.* The results are read after the addition of the salt solution. Optimum reading conditions in each laboratory should be determined by trial. The following points will be found helpful: (a) When utilising daylight for reading the tests, it is well to have but one source of light coming from a single window immediately in front of the reader. It will be found satisfactory to shade the upper and lower portions of the window, narrowing the source of light to a section several feet in height. Light from any other windows near the reader should be dimmed by lowering the window shades. (b) When holding the rack in front of the exposed section of the window, the definitely positive and the negative reactions are readily differentiated without lifting the tubes from the rack. (c) In case of weak reactions, examine each tube individually, lifting it several inches above the eye level and slanting it until the fluid is spread into a thin layer. The precipitate will then become readily visible.

Those preferring magnification will find the microscopic mirror helpful. Place mirror on reading table with concave surface upward. Hold the tube in slanted position two to three inches above the mirror and examine the image in the mirror. Both daylight and artificial light may be employed. One may also utilise an ordinary hand lens for reading the tests. A two- or three-fold magnification will be found satisfactory. Some workers prefer the use of a slit-light arrangement, the source of light being an electric bulb enclosed in a box which is provided with a narrow slit.

As far as possible, workers should limit themselves to one method of reading. The occasional use of magnification by readers who usually do not resort to it will be likely to affect the uniformity of

their reading scale. It should be emphasised that certain highly magnifying agglutinoscopes show particles in serum alone, and are thus unfit for use in this test. The magnification must be sufficiently low as to assure opalescent and clear-cut negative reactions, with entire freedom from visible particles.

8. *Interpretation of results.* A definite precipitate suspended in a clear medium is read +++++. Proportionally weaker reactions are read +++, ++, + and ± respectively. The *final results* is the average of the readings of the three tubes, as indicated in Table I.

Table I. OUTLINE OF KAHN TEST AND INTERPRETATION OF RESULTS.

	Tube No.	1	2	3	Completion of test.
Serum : Antigen suspension		3 : 1	6 : 1	12 : 1	Tests are shaken three minutes, 1 c.c. salt solution is added to first tube and 0.5 c.c. to other two tubes and results are read.
Antigen suspension c.c.		0.05	0.025	0.0125	
Serum (heated at 56° C. for 30 minutes) c.c.		0.15	0.15	0.15	

Interpretation of Results.

Reaction No.				Final result (average of reactions of three tubes)
1.	+++++	+++++	+++++ ¹	
2.	+++	+++++	+++++	+++++
3.	++	+++++	+++++	
4.	+	+++	+++++	+++
5.	—	+++	+++++	
6.	—	++	+++++	++
7.	—		+++++	
8.	—	—	+++	+
9.	—	±	++	±
10.	—	±	+	
11.	—	—	+	—
12.	—	—	—	

¹ Weakly potent sera show most marked precipitation in the third tube because a small amount of *reagin* reacts best with a small amount of antigen suspension, the relatively larger amounts of suspension in the first two tubes being inhibitory to precipitation.

Strongly potent sera show +++++ precipitation in each tube, but, owing to the different amounts of antigen suspension employed, the precipitates are of unequal bulk, being greatest in the first tube and least in the last tube.

In rare instances, an atypical reaction is obtained in which precipitation is marked in the first tube and weak or negative in the second and third tubes. In such a case, a quantitative test should be made and, if the result is twenty units or more, the qualitative reaction may be considered +++++; if less than twenty units, the results of the qualitative reaction should be averaged.

9. *Recording results.* Make a permanent record of findings in all tubes of each test at time of reading. Preferably, the tests should be read independently by two separate workers. When two workers are not available, the original reading should be checked by the same worker after a short interval.

10. *Procedure with less than three tubes.* If there is insufficient serum for the three-tube test, examine and report as follows: (a) If enough serum for two tubes, employ the lesser amounts of antigen suspension; report as a two-tube test. (b) If enough for one tube, employ the least amount of antigen suspension; report as a one-tube test. (c) If less than 0.15 c.c. serum is available, a one-tube test (micro test) may be made by employing 10 parts of serum to one part of antigen suspension: thus, if 0.05 c.c. of serum is available, it is employed with 0.005 c.c. of antigen suspension; report these as micro tests.

IV. Qualitative Spinal Fluid Process.

In this process, the greater part of the spinal fluid globulins is precipitated by means of ammonium sulphate and re-dissolved in an amount of normal saline equivalent to a tenth of the original spinal fluid volume. The concentrated globulin solution thus obtained is then tested with antigen suspension.

1. *Preparation of Globulin Solution.* (a) Centrifuge spinal fluid to render it free from cells and foreign particles. (b) Add 3 c.c. of the clear fluid to a conical-shaped 10 or 15 c.c. centrifuge tube. (c) To the same tube add 2 c.c. of a saturated solution of ammonium sulphate. (The ammonium sulphate must be of highest purity, such as Merk's Reagent or Baker's Analysed.) (d) Mix vigorously and place in 56° C. water-bath for fifteen minutes. (e) Centrifuge at high speed for fifteen minutes to throw down the precipitated globulins. (f) Remove with capillary pipette the supernatant fluid *as completely as possible*. (g) Add 0.3 c.c. normal saline to the precipitate and re-dissolve it by gentle shaking. This saline should be added by lowering the pipette close to the bottom of the tube to avoid washing down ammonium sulphate. The use of a 0.2 c.c. pipette is preferred for adding saline. With this pipette, the fluid is gently drawn up and expelled to assure the complete solution of the precipitate. This re-dissolved globulin solution is now ready to be tested with antigen suspension.

2. *Preparation of Antigen Suspension.* Mix salt solution with antigen in the same manner as for the test with serum, according to the required antigen titre for spinal fluid. The antigen suspension should stand ten minutes before its use in the test and should be used within thirty minutes.

3. *Measuring of Antigen Suspension.* With a 0.2 c.c. pipette graduated to 0.001 c.c., measure 0.01 c.c. of antigen suspension to the bottom of a test-tube.

4. *Measuring of Concentrated Globulin Solution.* Measure 0.15 c.c. of concentrated solution into the antigen suspension tube, using a 0.2 c.c. pipette. Shake tests vigorously for a few seconds to mix ingredients.

5. *Controls.* Include positive and negative spinal fluid controls; also observe each concentrated globulin solution to establish that it is free from foreign particles.

6. *Shaking.* After mixing the concentrated fluid with antigen suspension, shake tube vigorously for three minutes.

7. *Addition of Salt Solution.* Add 0.5 c.c. normal saline to tube.

8. *Reading of Results.* A definite precipitate suspended in a clear medium is read + + + +. Proportionally weaker precipitates are read + + +, + + and + respectively.

(The spinal fluid procedure may be carried out with 1.5 c.c. spinal fluid plus 1 c.c. saturated ammonium sulphate solution. Dissolve globulin precipitate in 0.15 c.c. normal saline (instead of 0.3 c.c.) and perform test with 0.01 c.c. antigen suspension.)

Highly Potent Spinal Fluid. It should be emphasised that highly potent spinal fluids give positive reactions directly without concentrating the globulins by means of ammonium sulphate. It is recommended, therefore, that spinal fluids from clinically diagnosed cases of neuro-syphilis be examined by mixing 0.15 c.c. fluid plus 0.01 c.c. antigen suspension, using the same antigen titre as for tests with serum. If the spinal fluid is definitely positive (+ + + +, + + + or + +) in the unconcentrated state, the reaction may be interpreted as + + + + in the qualitative procedure. If the reaction is very weak or negative (+, ± or —), the spinal fluid is examined in the usual manner, after concentrating the globulins.

METHOD⁷ USED BY PROFESSOR E. MEINICKE.

1. *Method:*

The M.T.R. (Meinicke Turbidity Reaction.)

2. *Principles:*

The organ extract employed is an alcoholic horse-heart extract highly diluted with alcohol, and to which balsam of tolu and benzoic acid are added. A dilution of this extract is prepared with saline. If this saline dilution is mixed with active sera, the liquid becomes turbid in the case of syphilitic sera, whereas with negative sera the liquid remains clear. Thus turbidity is the indicator in the method. In contradistinction to other flocculation reactions, active sera are used.

3. *Advantages:*

Great simplicity and speed. The sera are not inactivated. No incubator is required, as the reaction is carried out at room

temperature. No special optical instruments are needed for reading. This is done with the naked eye. The M.T.R. can easily be carried out in any small laboratory provided with a centrifuge.

4. The test is carried out at *room temperature*.

5. The results are read in *one hour* and again after a further hour. Thus the reading is completed in two hours.

6. Three test-tubes are required for testing one serum, *i.e.*, one for the control, one for the dilution of the strong extract, and one for the dilution of the weak extract.

7. In addition to the *pipettes* used for distributing the sera, the following are required: (1) one pipette for the original extract; (2) one graduated cylinder for the saline solution; (3) one pipette for distributing the prepared extract dilution, *i.e.*, three pipettes in all.

8. No *special apparatus* is needed for the test. Only a centrifuge is required for spinning the sera as in all serum reactions.

9. *Details of the technique:*

The technique for the Meinicke Turbidity Reaction (M.T.R.) is at present as follows:

Preparation of the extract. — The extracts, prepared as for the D.M. reaction, are diluted with 96 % alcohol in the proportion of 1 : 14 approximately, and balsam of tolu and benzoic acid are added. Extracts must be protected from light and kept at room temperature. In the test proper, one strong and one weak extract are used simultaneously.

Dilution of the extract. — The necessary quantity of extract (1 volume) is poured into a test-tube. Ten times this volume of 3 % saline containing crystallised soda 0.01 per cent is poured into a second tube. The two tubes are heated at 45° C. for five to ten minutes and their contents are then very rapidly mixed by first pouring the saline into the extract and then pouring the whole from one tube into the other, back and forth two or three times.

Sera to be tested. — The sera should be freed from all cellular elements, preferably by centrifuging. They are used in their *active* state and therefore must not be heated.

Test. — Of the freshly prepared extract which has been allowed to mature for five minutes, 1 c.c. is added to 0.2 c.c. of the serum to be tested. One drop of commercial formalin is added to the *control* tube. The tubes are left for an hour at the temperature of the well-warmed laboratory (about 20° C.).

Reading results. — Standing at a distance of about two or three metres from a well-lighted window, the worker looks at the window frame through the fluid. If the daylight is very poor, the reading is done by artificial light (19 candle-power). A suitable apparatus can be extemporised by means of a small box fitted with two electric bulbs. At the front end of the box an opening, 15 cm. high and 20 cm.

wide, is made and covered with a plate of ground glass, in the middle of which a large black cross is drawn. To read the results, the tubes are held at a distance of about 60 cm. from the box and the operator looks through them at the cross, which is seen more or less distinctly.

Interpretation of results. — When the serum is *negative*, the liquid is as transparent in the tube of the test proper as in the control tube. The cross is clearly outlined and black.

When the serum is *strongly positive*, the liquid in the tube of the test proper is *very turbid* and the cross is *no longer visible*.

A *weakly positive* reaction is recognised by a *slight turbidity* in comparison with the control tube: the cross, seen through the tube of the test proper, looks *indistinct with blurred outlines*. The difference between the aspect of the fluid in the tube of the test proper and in the control tube is always sufficient indication of the nature of the reaction.

In doubtful cases it is advisable, in addition, to carry out a Meinicke Micro-Reaction (M.M.R.). With this method the course of the reaction itself may be followed under the microscope, and impurities and infections of the sera, which may occasionally lead to false results in all syphilis reactions, are detected.

Untersteiner has published a useful method of applying the M.T.R. in cerebro-spinal fluid examinations.

Untersteiner mixes one part of M.T.R. extract with five parts of 1 % saline, after heating them to 45°; 0.5 c.c. of this mixture are added to 0.5 c.c. of active fluid and shaken. The test tubes are left at room temperature. The results are read in one hour by the hanging drop method as described for the Meinicke Micro-Reaction.

THE TECHNIQUE OF THE MEINICKE MICRO-REACTION (M.M.R.) is as follows:

Extracts and dilution of extracts. — The extracts are those used for the macro-reaction (M.T.R.), but containing rather less balsam. The dilutions are similarly prepared.

Active sera are used, very small quantities are required, and the blood can therefore be procured from the finger or the ear.

Test. — The extracts and sera are mixed by means of a *platinum loop* and not with pipettes as in the macro-reaction. A dilution of freshly prepared extract, matured for two minutes, is poured into a porcelain capsule. One drop is taken with a platinum loop 5.5 mm. in diameter. A drop of serum is then taken with another platinum loop, smaller than the preceding, *i.e.*, 2.5 mm. in diameter. The contents of the second platinum loop are then introduced into the lumen of the first loop, thus mixing the two liquids. A drop of the mixture is then taken with the smaller loop and deposited on a cover-slip, which is then covered with a concave slide. The preparation is left for 45 to 60 minutes at laboratory temperature (20° to 22° C.).

Reading results. — The preparations are read with a microscope fitted with a weak eye-piece and a strong dry objective — *e.g.*, LEITZ, eye-piece 1 or 2, objective 6 or 7. The various parts of the preparation must be carefully examined.

When the reaction is *negative*, nothing can be seen, or only a number of moving specks at the limit of visibility. *Strongly positive* reactions are characterised by the presence of large clumps which by their weight sink to the bottom and must be sought in the lower strata of the fluid.

When the reactions are *weakly positive*, larger or smaller clumps can be seen, of which the larger sink to the bottom of the preparation.

These two reactions, the M.T.R. and the M.M.R., are *extremely simple and very easy to read*. Any worker, however little practised in handling pipettes, can conduct these tests. He requires no special instruction for the purpose, but needs only a capacity of appreciating degrees of turbidity such as is possessed by every doctor familiar with testing the urine for albumin. For the micro-reaction, an ordinary knowledge of microscopic technique is required.

Neither method requires the use of an incubator or a regulated water-bath for serum inactivation.

The micro-reaction can even be conducted without a centrifuge: the blood corpuscles and fibrin are merely allowed to deposit, when the clear, supernatant serum can be used. Only a few glass vessels, the platinum loops, and a microscope are essential.

METHOD USED BY PROFESSOR R. MÜLLER.

1. *Method:*

Müller Conglomeration Test (Müller Ballungs-Reaktion).

2. *Principles:*

Alcoholic ox-heart extract, to which a fairly high proportion of cholesterin has been added, is concentrated in the water-bath and then stored in this concentrated form (conglomeration reagent). For use it is warmed and diluted in two stages and in fixed proportions with 0.9% saline and then placed to mature for several hours in a thermostat at 56°C. With positive inactivated syphilis sera, this antigen causes the formation of a single freely suspended globular complex of white or yellowish-white gelatinous appearance, generally with a denser nucleus which is rather dark in colour and has a delicate nebular outer zone, whereas in negative cases the whole suspension has a perfectly clear colloidal appearance. In very rare cases of grave non-syphilitic disease of various kinds, crumb-like precipitates are formed which can readily be distinguished from conglomeration.

3. *Advantages :*

The method is ^{is}ac^{is}sitive to a very wide range of cases of syphilis, with marked sp^{is}ec^{is}ificity. Positive reactions are quite clearly defined. Lends itself to an accurate quantitative work and can be used for testing the cerebro-spinal fluid.

4. *Temperature :*

37° C.

5. *Reading results :*

The first reading is taken after nine hours in the incubator.

A second reading after a further fifteen hours at room temperature.

6. *Number of tubes required for one serum test :*

Three.

7. *Number of pipettes required :*

One dropper for delivering the various sera ; one pipette for saline ; one pipette for preparing the antigen to be used ; and one for delivering the prepared antigen into the individual test-tubes.

8. *Other apparatus required :*

Test-tubes for the test proper, a small number of test-tubes for heating the concentrated reagent and maturing the diluted antigen ; two beakers for diluting the antigen. An inactivating water-bath (56° C.) ; an incubator (37° C.) ; a thermostat (56° C.) for maturing the antigen, or, failing the thermostat, a metal box with closely fitting lid which can be immersed in the 56° C. water-bath and inside which the physical conditions of a dry thermostat obtain.

9. *Details of the technique :*

The principle recommended for the preparation of the Wassermann antigen (namely, concentrating cholesterinised ox-heart extract and maturing for several hours the colloidal antigen obtained after dilution with saline), has been adhered to in the preparation of antigen for the conglomeration test. As ox-heart muscle varies considerably in suitability, and as a great deal depends on very accurate adjustment of the extract with cholesterin, and as, moreover, it is difficult to obtain the exact degree of concentration in the laboratory, it is necessary to obtain selected and correctly cholesterinised, ready-concentrated antigens from a distributing centre ("Ballungsreagens" Schering-Kahlbaum, Berlin).

The preparation of colloidal antigen from the conglomeration reagent is described below, in detail.

Directions for the preparation of the colloidal conglomeration antigen. — The bottle is vigorously shaken and 8 c.c. (or, if smaller quantities are required, 4 or 2 c.c.) of the reagent are poured into a moderately long test-tube, which is then well corked and heated for half-an-hour at 56° (water-bath). The heating causes any flocculated lipid particles to re-dissolve. The reagent is now diluted with an accurate

0.9% solution of sodium chloride (the figures given here apply to Kahlbaum's NaCl crystals). 5 c.c. of saline are poured into a small beaker (I) about 45 mm. in diameter, and 50 c.c. into a second beaker (II). If, in the case of certain antigens, a small modification in the degree of dilution should prove desirable, a slip bearing a notice to that effect would be enclosed in the package. The saline in both beakers must be raised to a temperature of 17° C. (accurately to within about half a degree). The mixing must be carried out as follows: the reagent, taken from the water-bath, is poured quickly and suddenly into beaker I, the test-tube is laid aside and beaker II seized with the same hand and its contents added equally quickly and suddenly to the first mixture. This method provides the simplest means of restricting the maturing period to about one and a-half seconds, which is the optimum time for the dispersion of the colloidal antigen. The colloidal solution obtained in this manner is poured into test-tubes 18-20 mm. in diameter, with walls $\frac{3}{4}$ mm. thick, which, when well sealed with a rubber stopper, are placed, to further mature the solution, for twenty-four hours in a 56° C. incubator (*not* water-bath). The matured colloidal antigen, when kept for about two days at room temperature, scarcely loses any of its activity. If the mixture has been correctly prepared, the conglomeration antigen should form a uniform colloidal suspension, when matured.

8 c.c. of original extract is enough for the quantitative examination of about 40 cases. If the number of cases to be examined is smaller, the same process of dilution can be carried out with 4 or 2 c.c. of original extract. The quantities of saline are then correspondingly smaller, *i.e.*, 2.5 and 1.25 c.c. respectively in beaker I and 25 and 12.5 c.c. respectively in beaker II.

In order to obtain good conglomeration antigen, the instructions given here must be strictly adhered to, more especially as regards the use of absolutely pure distilled water, concentration and temperature of the saline, interval between the two stages of dilution, period of maturing, size and sealing of the test-tubes, temperature of the incubator, etc. All glass vessels must be scrupulously cleaned, (the final rinsing should be made in distilled water).

Directions for carrying out the conglomeration test. — The sera are inactivated for half-an-hour in a 56° C. water-bath. For the examination of one specimen, three carefully cleaned small test-tubes (Vidal tubes) of about 8 mm. internal diameter are required. Into these are delivered respectively 0.15 c.c. (3 drops), 0.2 c.c. (4 drops) and 0.25 c.c. (5 drops) of the inactivated serum (the volume of each drop must be exactly 0.05 c.c.¹); 0.05 c.c. of the ripened antigen is then added to each of these tubes from the antigen tube, which must be shaken twice before opening, so that the contents may be uniform. The contents of the Vidal tubes are well mixed by shaking for a short

¹ If in an exceptional case owing to lack of serum, one dose has to be omitted, it is best to leave out the middle tube (4 drops). A fairly accurate quantitative reading can then still be obtained.

time. The tubes are then placed, uncorked, in the 37° C. incubator for from seven to nine hours, after which the first reading is taken. The reaction is continued for a further nine to fifteen hours at room temperature, after which the final reading is taken.

Cerebro-spinal fluid can be tested in the same manner, but with different doses. The fluid should also be inactivated for half an hour.

The doses are as follows:

Fluid:	0.05 (1 drop)	0.1 (2 drops)	0.15 (3 drops)
Antigen :	0.3	0.3	0.3
Fluid:	0.3 (6 drops)	0.45 (9 drops)	0.9 (18 drops) ¹
Antigen:	0.3	0.3	0.3

N.B. — In the examination of spinal fluid the antigen must not be dropped, but measured from a pipette.

Period of incubation in thermostat and at room temperature, as for serum examination.

Reading results. — With correctly prepared antigen, a positive reaction produces a globular mass of white or yellowish-white gelatinous appearance, generally with a denser nucleus, which is rather dark in colour and has a delicate nebular outer zone. This mass is freely suspended in the centre of the column of liquid. If the preparation of the antigen has not been perfect the conglomeration will often appear opaque and crumbling. The results are best expressed in terms of intensity of reaction.

The strongest reaction (IV) is that in which conglomeration already occurs at incubator temperature with the smallest dose of serum; the weakest positive reaction (I) is that in which conglomeration does not set in until the mixture has stood at room temperature, and then only with the largest dose of serum.

Grade of Reaction	Results at :					
	7-9 hours at 37° C.			9-15 hours at room temperature.		
	0.15	0.20	0.25	0.15	0.20	0.25
I	—	—	—	—	—	+
I-II	—	—	—	—	+	+
II	—	—	—	+	+	+
II-III	—	—	C ²	+	+	+
III	—	—	+	+	+	+
III-IV	—	+	+	+	+	+
IV	+	+	+	+	+	+

Interpretation of Results. — The M.B.R., like the B.-W. and all flocculation tests, may also, in exceptional cases of *Tbc. florida*, acute suppuration, etc., give “unspecific” positive results where no syphilis is present. Leaving out of account the rarity of such unspecific results (3 to 4 per cent in *Tbc. florida*), the quantitative method of the M.B.R. admits of differentiation, in so far as reactions which

¹ Instead of using 0.9 of fluid, the quantity of fluid and extract can, for the sake of economy, be proportionately reduced (0.6 : 0.2 or 0.3 : 0.1).

² Clouding.

show a positive result at the *early reading* (degrees of intensity IV, III and II-III) unquestionably indicate syphilis, whether the patient is also suffering from one of the above-mentioned diseases or not (except malaria and leprosy). On the other hand, the results of the *late reading*, especially degrees of intensity I and I-II — like a weakly positive B.-W. — must only be considered as indicative of the presence of syphilis if the patient is not simultaneously suffering from some *grave non-syphilitic disease*.

At all events, it would be advisable, before evaluating such a result, to repeat the test after a short lapse of time. The highest degree of intensity at the late reading (degree of intensity II) is not so *absolutely* certain an indication of syphilis as the results of the early reading, but a reaction as strong as this is so rare in the case of even the most serious non-syphilitic diseases that, in establishing a diagnosis, result II would, under any circumstances, have to be given due consideration. Therefore, in the absence of any grave non-syphilitic disease, the presence of syphilis is indicated with complete certainty by result II and with the *highest degree of probability* by the weakest result (I). The latter result is frequently obtained in cases of *syphilis latens*, and also tabes and sclerosis, when all other reactions are negative.

Unspecific Reactions. — In addition to the very rare unspecific conglomeration of the lowest degree of intensity (I) already mentioned, cases of disease in which the serum is said to be in a labile condition, may quite exceptionally show precipitates or larger clumps; these appearances, however, are so distinctive that they cannot be mistaken for positive clotting reactions.

It is characteristic of unspecific reactions of this kind that the small dose of serum (especially 3 drops) often reacts more strongly than the larger doses.

METHOD USED BY DR. M. NAGAYO AND DR. NOBECHI.

THE MURATA METHOD, A MODIFICATION OF THE SACHS-GEORGI REACTION.

1. *Method used:*

Murata method.

2. *Principles:*

Formation of a precipitation layer, when the antigen solution is superposed upon the patient serum.

3. *Advantages:*

- (a) The simplest method of all.
- (b) Consumption of least time for test.
- (c) Smallest quantity of serum required for the test.
- (d) Specificity of this test is demonstrated by the curves observed with sera of leprosy or cancer patients.

4. *Temperature:*

37° C.

5. *Reading results:*

After forty-five minutes with clear sera, and after three to four hours with chylous sera (turbid).

6. *Number of tubes required:*

One tube per serum.

7. *Number of pipettes required:*

(a) One pipette of 1 c.c. for antigen.

(b) One capillary pipette for the delivery of sera, and another for the distribution of the antigen solution.

8. *Other apparatus required:*

(a) Two tubes of about 2.5 cm. diameter for the dilution of antigen.

(b) One graduated cylinder for the measurement of 10 c.c. saline solution to make the antigen dilution.

(c) Test-tube racks.

(d) A blackboard to facilitate reading of test.

9. *Other details of the method:*

Murata's method is a modification of the Sachs-Georgi reaction. It consists in a ring test with the cholesterinised crude ox-heart extract. The specific features of the test are as follows:

As well known and especially well elucidated by Neukirsch's thorough studies, the cholesterinised extract suffers from the effect of reaction temperature. If, however, the cholesterin content is reduced to a certain degree in proportion to the amount of extract, a phase is reached when the temperature effect is insignificant and yet the reaction is sufficiently intensified by the cholesterin fraction. Using this phase, Murata made the antigen sufficient for the reading of the reaction after forty-five minutes at room temperature. Murata's test is thus done without the use of an incubator.

The antigen dilution to be used as the reagent in the Murata test appears water-clear in the slender reaction test-tubes, whilst it facilitates the recognition of the ring formation in positive cases. Murata's test owes this advantageous feature chiefly to the low content of cholesterin in the antigen in proportion to the amount of extract.

The most important feature of the Murata test is its specificity to syphilis, which is based upon experimental evidence. After five years' studies on the serodiagnosis of syphilis, especially on the positive reactions with non-syphilitic cases, Murata and Tamiya, in our institute, came to the conclusion that the variation in the proportion of cholesterin to extract not only changes the sensitiveness of the antigen, but also alters its specificity.

The ring tests are based upon the result of the whole range of the gradually varying phases of mixtures of the serum with antigen.

Therefore, the contact precipitation ring formation cannot be missed in the case of a positive serum, no matter what is the optimal phase of mixture. The ring test overcomes the difficulty of zone phenomena.

The interpretation of the results of the ring test is usually much easier than that of flocculation tests. Furthermore, the performance of the test is the simplest and the most economical of time, as arbitrary amounts of serum and antigen are applied for individual tests, and such steps as those of the measurement of the reagents for individual tests or the dilution of each serum are saved. As it is a highly sensitive reaction, the results can be read within a short duration of time with the thin dilution of antigens. In the case of the flocculation tests, one has to use a thick antigen dilution such as Kahn's if the reaction time has to be shortened. Nobecki found that Kahn's antigen diluted 100 times is just sufficient for the Murata test, as far as sensitiveness is concerned.

The disadvantage of the ring test is the occurrence of the false ring, which sometimes makes the reading of the reaction difficult. The false ring, however, is mostly formed in cases with hæmolysed or chylous sera; and, if the precautions, described later in the technique concerning the sera, are observed, it will be easily avoided.

The established technique of the Murata method contains special features in the technique of the precipitation test. The detailed method is given below.

TECHNIQUE.

I. Apparatus required.

1. Pipette of 1 c.c. capacity . . . to measure the antigen.
2. Capillary pipette. to distribute the antigen dilution and the sera.
3. Mouthpiece with rubber tubing or rubber cap . . . to operate the pipettes.
4. Two test-tubes of *circa* 2.5 cm. diameter one to contain the antigen and the other the saline solution, for the preparation of the antigen dilution.
5. Test-tubes of *circa* 0.5 cm. diameter to carry out the tests therein.
6. Test-tube rack.
7. Small blackboard to facilitate the reading of the result, it is held behind the test-tube rack against the window.

II. The Sera.

The sera are to be inactivated by placing them for thirty minutes in a water-bath at 55° C. Before the application of the test, they must be kept standing at room temperature at least thirty minutes after the completion of inactivation. The same precaution must be observed when the tubes have been kept in the ice-chest after inactivation.

Chylous and hæmolysed sera sometimes make the reading of the results difficult. It is therefore advocated that the blood be taken before a meal, preferably before breakfast. Sera in which spontaneous clotting occurs are to be centrifugalised or kept standing before inactivation, in order to remove blood corpuscles.

III. *The Antigen.*

(1) *Preparation of the antigen.* — The fat-free part of ox-heart muscle is stored in the ice-room overnight, and is then finely mashed by passing it through a mincing machine several times, the exuding fluid being discarded. 500 c.c. alcohol (*circa* 96 per cent) is added to 100 gr. of mashed muscle. The resulting suspension of mashed muscle is allowed to stand at room temperature for a week, and is occasionally shaken. It is afterwards put in the ice-chamber and, after being stored there for two days, it is filtered cold. To the filtrate, two parts of alcohol are added. The diluted extract is filtered, and stored at room temperature for a few months and filtered again. Thus the final extract is made up, ready for cholesterinisation.

The 1% alcoholic solution of cholesterin is made from the preparation of Merck, Darmstadt. This is added to the extract in the proportion indicated by the result of titration for sensitiveness.

(2) *Standardisation of the antigen.* — The cholesterin will precipitate if it be diluted with the saline solution alone, whereas the addition of a certain amount of extract does not cause the precipitation of the cholesterin in the dilution with the saline solution. The more extract is added the greater stability of the cholesterin solution results. The extract therefore acts as a protective colloid upon the cholesterin.

A definite amount of the cholesterin solution is put in several test-tubes, and to these varying amounts of the extracts are added; 1 c.c. of each mixture is placed in a test-tube of *circa* 2.5 cm. in diameter and is diluted with 10 c.c. of the saline solution from a test-tube of the same diameter. The dilution is carried out with as rapid a movement as possible and in a definite way. After ten minutes' interval, the test is performed with each of the dilutions, using both normal and syphilitic sera. After observation of the results at the end of forty minutes at room temperature, the mixture with the requisite sensitiveness is fixed upon as the standard antigen. In the case of Murata's own standard antigen, the border-line between normal and syphilitic is fixed to coincide with that of the Taniguchi's Wassermann test, *i.e.*, the Wa — sera with the named test will not cause precipitation rings but the \pm sera will form faint rings.

To titrate the new antigens, the mixtures of the cholesterin solution with varying amounts of new extract are prepared and diluted with saline solution as described above. A positive serum, preferably with a clinical diagnosis of syphilis, is taken and diluted with saline solution five times, ten times, fifteen times, etc. Applying these dilutions of the serum, the sensitiveness of the mixtures of the cholesterin solution with the new extract as antigen is compared

with that of the standard antigen. The mixture showing exactly the same sensitiveness is taken as suitable for the antigen. One part of cholesterin solution to 17.5, 18, 18.5, or 19 parts of extract is usually found to be the correct proportion of the mixture in the preparation of the standardised antigen. If two mixtures with different proportions are equally sensitive, the one which contains the greater proportion of extract is selected in order to be as much farther away as possible from the phase to cause the non-specific reactions. If a certain mixture is found a little too sensitive, slight reduction of the extract (the protective colloid) in this method will be sufficient to adjust its sensitiveness, and *vice versa*. By this means the delicate adjustment of the sensitiveness of the antigen is done with ease.

1 c.c. of the antigen is measured into a test-tube of *circa* 2.5 cm. diameter, and 10 c.c. of the physiological saline into a second tube of the same diameter. The latter is added to the former with as rapid a movement as possible and the mixture is returned to the second tube. The antigen dilution thus prepared is ready for use in the test after standing ten minutes, and may be used within an hour after preparation. The "made-up" antigen dilution has a faint opalescence, but in the slender test-tubes it appears quite clear. Should it appear opaque, it is not suitable for use. Unclean glassware is in most instances responsible for abnormal cloudiness.

IV. The Test.

Into a test-tube of *circa* 0.5 cm. diameter, a quantity of inactivated serum is introduced, so as to reach the height of *circa* 1.5 cm. The antigen dilution is then superposed upon the serum layer. For the purpose of obtaining the clear-cut contact surface between the two media, the following precautions are taken. *At the end of distribution of serum*, the inner wall of the test-tube over the serum layer must be moistened by a circular motion of the tip of the capillary pipette, and then the latter is withdrawn along one side of the test-tube so as to leave a wet line. The last drop of the serum is let run down along the wet line to open the path for the antigen. The delivery of the antigen is begun with a drop of antigen slowly running down along the wet line described above. After ascertaining that the first drop of antigen has reached the upper surface of the serum layer, a few more drops are added with the same precaution, and then five to ten more drops in succession. After distributing the antigen, it should be noted that the contact surface in each tube is sharp. As far as our experience goes, this drop method is the best and easiest one for successful ring tests and the mode in which the first drop of the antigen is introduced is the key to success.

It is not necessary to change the capillary pipette for each serum; but, after one serum is distributed, the tip of the pipette is wiped with a piece of gauze, the inside of it is rinsed out with saline solution a few times and the adhering trace of saline solution wiped off with another piece of gauze, and the pipette is ready for use for the second serum.

V. *Reading the Result.*

The results are read at the end of forty-five minutes' standing at room temperature. If a white ring appears at the contact surface the reaction is positive, and if this is not the case the reaction is regarded as negative. According to the intensities of the white ring formation, the reactions are recorded as $+++$, $++$, $+$ and \pm . With some sera the false ring is formed, which frequently interferes with the reading. The true ring as well as the false one appears on the contact surface; but, whilst the former is caused in the antigen layer and its lower-limit line is sharp, the latter is formed in the serum layer and its upper-limit line is clear-cut. In doubtful cases, at the second reading after two to four hours, the true ring will increase its density and width, whereas the false will remain as it was or even become less marked.

Reading must be done sitting at a light window without shade, with the blackboard held behind the test-tube rack. Controlling the light by lifting or lowering the blackboard helps sometimes in the recognition of a weak reaction. The observer's eyes must be on the same level as the contact surface.

No control system is required, as the antigen is very accurately standardised.

METHODS OF PROFESSOR H. SACHS AND DR. E. WITEBSKY.

A. SACHS-GEORGI REACTION (LENTOCHOL).

1. *Method employed:*
Lentochol reaction.
2. *Principle:*
Direct flocculation of cholesterinised extract.
3. *Advantages:*
Simple flocculation and a high degree of specificity for syphilis.
4. *Temperature:*
37° C.
5. *Reading results:*
Eighteen to twenty-four hours.
6. *Number of test-tubes required for one serum test:*
Two to three test-tubes (naturally, for quantitative purposes more are required).
7. *Number of pipettes required:*
One pipette for each serum, one for saline, and one or two pipettes for extract dilutions.
8. *Apparatus required:*
An agglutinoscope for reading results is useful though not absolutely necessary.

9. Other details of technique:

I. *Extracts.* — Cholesterinised organ extracts are used for the flocculation reaction. The choice of the organ and of the method of extraction are of secondary importance, but the extracts must be sufficiently sensitive and, as far as possible, specific for syphilis. These requirements can only be fulfilled after extensive empirical tests have been carried out with each of the extracts.

Professor Sachs supplies suitable cholesterinised extracts (Institute for the Experimental Study of Cancer, Heidelberg). These extracts are made from ox-heart to which cholesterin has been added.

They are diluted immediately before use with five times their volume of physiological saline. This dilution is made in two stages. One part of cholesterinised ox-heart extract is quickly mixed with one part of saline. The mixture is shaken for an interval which varies from ten to twenty seconds, after which the four further parts of saline are quickly added. It is extremely important that the instructions with regard to the interval between the first and second additions of the saline should be strictly followed. These instructions are specially given for each individual extract.

II. *Patient's serum.* — Patient's serum is heated, immediately after the blood has been taken, for half-an-hour in the water-bath (inactivation at 55° C.). As with all methods of serological diagnosis of syphilis, the serum should, as far as possible, be free from pigment and should be tested as fresh as possible.

III. *Saline.* — A solution is prepared of chemically pure 0.85 % sodium-chloride in *distilled* water. The solution must be clear. If it contains floating particles of cotton wool or other substances, it must be filtered through ordinary filter paper.

IV. *Technique.* — The flocculation reaction is carried out in tubes 100 mm. in length and 15-16 mm. internal diameter. The walls of the tube must not be thick, for thickness would render the reading of the results difficult or even impossible.

Each serum is tested with two extracts. As a control, each serum is mixed with a corresponding quantity of alcohol at 96 per cent. The serum controls are actually put up as follows: 0.5 c.c. of a 1-in-5 dilution of serum mixed with 0.25 of a 1-in-6 dilution of 96 per cent alcohol in saline. Three tubes are therefore required for each serum, preferably placed one behind the other in the rack. The various patients' sera are placed one beside the other. In this way three parallel rows are obtained:

- A. The first principal test with Extract A.
- B. The second principal test with Extract B.
- C. The control with alcohol.

Inactivated patient's serum is diluted 1 in 5. The figures given below refer to tests made with 0.5 c.c. of a dilution of serum 1 in 5 and with 0.25 of extract diluted 1 in 6. These quantities may be doubled.

When working with 0.5 c.c. of a dilution of serum 1 in 5 there are two alternatives:

- (a) Either 1.2 c.c. of physiological saline is placed in the tubes in row C, and a 1-in-5 dilution is obtained by adding 0.3 c.c. of inactivated patient's serum, 0.5 c.c. of the solution thus obtained being then delivered to each tube in rows A and B;
- (b) Or 0.1 c.c. of inactivated patient's serum is placed in each tube in rows A, B and C, and a 1-in-5 dilution is obtained by adding 0.4 c.c. of saline.

In either case, at the ends of rows A and B there must be one tube containing only 0.5 c.c. of saline (extract control).

After setting out the sera in this way and entering the particulars in the records, the extracts are added. For this purpose 0.25 c.c. of extract dilution prepared in the manner indicated above is added to each tube in rows A and B immediately after preparation.

0.25 c.c. of a 1-in-6 dilution of alcohol (96 per cent) with saline is added to each tube in row C (serum control).

The tubes are now shaken one by one, and then all together in the rack, which is placed in the incubator. This must be kept at a constant temperature of 37° to 37.5° C.

V. *Reading results.* — Results are read:

- (a) After four to five hours in the incubator;
- (b) And after eighteen to twenty-four hours in the incubator.

The second reading is decisive irrespective of the earlier reading. In the majority of cases the result can be gauged at the earlier reading. Nevertheless, the second reading cannot be dispensed with on account of the rare cases in which the reaction is delayed or in which labile sera may produce a reversible flocculation which will have disappeared by the time the second reading is taken.

After shaking the tubes for a few seconds, the reading is taken with a Kuhn and Woihe agglutinoscope. These agglutinoscopes can be obtained from Paul Altmann, Luisenstr., Berlin, N.W., and from F. & M. Lautenschläger, Luisenstr., Berlin, N.W. The agglutinoscope must be adjustable to suit the observer, *i.e.*, it must be possible to alter the distance between the lens and the tube so that the flocculi are perfectly visible. It is easy to see whether the apparatus is serviceable by taking the reading with the naked eye in doubtful cases. Where there is even a slight reaction it is possible to see the flocculi with the naked eye; the observation can then be made much more accurately with the help of the agglutinoscope.

Specific flocculi can be distinguished by the fact that they are regularly distributed over the whole field of vision. They may range in size from very small granules to large masses. Irregular, doubtful-looking granules should not be interpreted as a positive flocculation. Obviously, a result must not be considered positive if flocculation is present in row C (serum control) and in the end tubes in rows A or B (extract control). If flocculation is present in the tubes at the

end of rows A and B, the inference is that the extract or the extract dilution is unsuitable for use in the test. If flocculation is present in a serum control tube (row C), the result must be rejected on account of auto-flocculation. In such a case, an attempt must be made to clarify the serum by means of centrifuging or filtration, or a fresh blood specimen must be taken from the patient.

The result is recorded in the usual approximate manner: a positive reaction being indicated by a larger or smaller number of “+” signs according to the degree of flocculation, a doubtful reaction being indicated by “±” and a negative reaction by “—”.

VI. *Advantages of the flocculation reaction.* — The advantages of the flocculation reaction when correctly carried out are, according to personal experience, the following: The reading of the results is easy, sensitiveness is sufficient, and the danger of a non-specific lability reaction is reduced to a minimum as compared with other serological tests for syphilis.

VII. *Sources of errors.* — To avoid errors it is necessary:

- (a) To use cholesterinised extracts of irreproachable quality and to adhere to the prescribed interval between the two dilutions;
- (b) To use an absolutely pure saline solution of 0.85 % (purest sodium chloride, distilled water);
- (c) To keep the incubator temperature constant;
- (d) To make sure that the agglutinoscope is serviceable (suitable distance between the lens and the tube).

VIII. *The quantitative flocculation test.* — The flocculation test may equally well be carried out quantitatively with decreasing amounts of serum on the same lines as those set out above. The total volume must, of course, be the same in all the tubes. By a quantitative test on these lines, it is undoubtedly possible to determine up to a certain degree the reacting power of a serum. But it is doubtful whether a quantitative test — always somewhat complicated — can enhance the practical value of serological tests. It is true that in rare cases a positive reaction may be adduced which has been masked by inhibition through excess (where the quantity of serum generally used was too large). The quantitative method entails no modification of the technique; it merely takes advantage of possibilities inherent in the process.

B. CITOCHOL REACTION.

1. *Method:*

Citochol Reaction.

2. *Principles:*

Rapid flocculation of concentrated cholesterinised extracts.

3. *Advantages:*

Simple technique, rapid results (without special apparatus), comparatively high specificity, greater sensitiveness than with the Lentochol Reaction.

4. *Temperature:*

Room temperature.

5. *Reading results:*

After half an hour.

6. *Number of test-tubes required:*

Two to three (according as one or two extracts are used).

7. *Number of pipettes required:*

One pipette for every serum. Also one pipette for saline, and one or two for extract dilutions.

8. *Other apparatus:*

None (possibly an agglutinoscope).

9. *Further particulars:*

Alcoholic benzoin solutions have proved comparatively unstable, and consequently must be prepared at frequent intervals; therefore, the principle of plain cholesterinised ox-heart extract first laid down by Sachs and Georgi has been reverted to in more recent experiments, in which some of the measures adopted in Kahn's carefully modified method have been taken into consideration. This method secures rapid flocculation. The interaction of a low dilution of concentrated cholesterinised extract with serum in strong concentration plays an important part in the production of a quick result. As in Bruck's method, a suspension of precipitated particles of extract is used instead of extract emulsion.

We tried concentrating our cholesterinised extracts for the Sachs-Georgi Reactions by steaming and using them in this highly concentrated form, with the result that *rapid flocculation could actually be obtained*.

The next step was to evaporate alcoholic ox-heart extract prepared according to the old method (*i.e.*, by the extraction of one part by weight of the moist substance with five parts by volume of alcohol), and to re-dissolve the triple concentration in alcohol. To these concentrated extracts cholesterin is added so as to obtain a cholesterin content of 0.3 to 0.6 per cent according to the nature of each individual extract. These cholesterinised ox-heart extracts are called *Citochol* extracts (citcholesterin), because they are used in the rapid reaction which will be described below, and we call the resulting method of flocculation the "*Citochol Reaction*" in contradistinction to the Sachs-Georgi Reaction, which, because of its slow reaction time, we call the "*Lentochol Reaction*".

The *Citochol Reaction* is carried out as follows:

1. The *Citochol* extracts are quickly diluted by mixing one part of extract with two parts of 0.85 % saline. The 1-in-3 dilution prepared in this way is left standing at room temperature for ten minutes before use.

2. For carrying out the *Citochol Reaction*, 0.1 c.c. of serum inactivated for thirty minutes at 55° C. are mixed with 0.05 c.c. of the

diluted extract. The mixture is then left standing at room temperature for half an hour, after which 0.5 c.c. of 0.85 % saline are added.¹

Thirty seconds' shaking may be substituted for the half-hour standing at room temperature.

3. The reading follows immediately, either macroscopically or in an agglutinoscope (possibly also as a turbidity reaction).

4. The Citochol Reaction can be carried out simply as a micro-reaction. For this purpose one to two parts of inactivated sera are mixed with one part of extract dilution on a slide or in a watch-glass or capsule and rapidly and evenly mixed. The result can be read on the slide after the lapse of a few minutes like a bacterial agglutination or a blood-group determination.

So far, results obtained by this procedure have been favourable, but there is the same drawback as in all rapid reactions, viz., under given circumstances unspecific results are obtained more frequently, though there may be increased sensitiveness.

This differential factor ought to be taken into account in the sero-diagnosis of syphilis by the flocculation of cholesterinised extracts; the Lentochol Reaction possesses a very high degree of specificity, but a lesser degree of sensitiveness. The Citochol Reaction is more sensitive, but does not perhaps possess an equally high degree of specificity.

METHOD USED BY DR. K. NOREL.

THE SIGMA (Σ) REACTION.

The Sigma Reaction has the following advantages:

- (1) Simple technique;
- (2) The results are expressed in Sigma units so that different tests are comparable;
- (3) When using standardised antigen and a standard flocculation test the reaction can be reproduced by different workers at different times in different places.

The original technique of Professor Dreyer and Dr. Ward at Oxford (1921) has been modified and simplified in the Danish State Serum Institute in Copenhagen; it is given in detail below.

With this modification, over 40,000 sera have been tested in Copenhagen, the Wassermann Reaction being performed simultaneously. It was found that the Sigma Reaction was equal to the Wassermann test in sensitiveness and the decrease in strength of the Sigma Reaction followed in a satisfactory manner the clinical picture during treatment.

¹ It is advisable to set up as controls parallel rows in which the extract dilution is replaced by a corresponding dilution of alcohol. Formalin controls are also useful.

A. Reagents and Apparatus required.

1. Antigen (alcoholic extract of calf's heart).
2. Alcoholic solution of cholesterin, 1 per cent.
3. Normal saline solution.
4. Stands with one row of holes.
5. Agglutination dwarf test-tubes, internal diameter 5.5 to 6.2 mm., external diameter 6.6 to 7.2 mm., length 58 mm., conical point, broad collar.
6. Graduated measuring cylinders: (a) to contain 25 c.c., height 11 cm., inside diameter 2.2 cm.; (b) to contain 100 c.c., height 16 cm., inside diameter 3.5 cm.
7. Bigger's dropping apparatus.
8. 0.5 and 1 c.c. pipettes, graduated in hundredths of 1 c.c.
9. A hand-lens magnifying six times.
10. A special artificial light apparatus (Sigma agglutinoscope).
11. A water-bath (54° C.) for inactivation of the sera.
12. A dry-air incubator (38.5° C.) for the incubation.
13. Rubber-stoppered tubes for collecting blood samples.

B. Technique (Elaborated by Norel, The State Serum Institute, Copenhagen).

The blood samples must be drawn aseptically; 2 to 3 c.c. blood (= 1 c.c. serum) is required. The serum is pipetted into sterile dwarf test-tubes plugged with cotton-wool (not with a rubber or cork stopper). The serum is heated for 90 minutes in the water-bath (54° C.) The inactivated serum is pipetted into seven to ten of the conical test-tubes according to the following table:

Tube No	1	2	3	4	5	6	7	8	9	10
Pure serum.....	0.4	0.2	0.1	0.05						
Serum dilution 1:10.....					0.2	0.1	0.05			
Serum dilution 1:100								0.2	0.1	0.05
Normal saline.....			0.1	0.15		0.1	0.15		0.1	0.15
α -Suspension	0.1									
β -Suspension . . .		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
(Degree of dilution)	1:1 $\frac{1}{4}$	1:2 $\frac{1}{2}$	1:5	1:10	1:25	1:50	1:100	1:250	1:500	1:1000

The two serum dilutions are prepared in two dwarf test-tubes, containing 0.9 c.c. saline; into the first tube is pipetted 0.1 c.c. serum; and into the second 0.1 c.c. of this 1-in-10 dilution.

The *antigen* is employed in two different dilutions: α -suspension and β -suspension. The suspensions must be prepared immediately or only about half an hour before the tests.

2 c.c. antigen is mixed with 0.2 c.c. cholesterin solution; this cholesterinised antigen is emulsified with saline by the help of Bigger's dropping apparatus in the following way: 1 c.c. cholesterinised antigen is poured into a measuring cylinder placed under the dropping tube (the tube is made of a piece of capillary tubing, 6 mm. external diameter, 0.5 mm. internal diameter); the height from the bottom of the cylinder to the point of the dropping tube is 36 cm.; the drops have a size of about 0.1 c.c. each; 34 c.c. must drop off in the course of nine minutes. The two antigen dilutions are:

α -suspension: Into 1 c.c. cholesterinised antigen is dropped $10^{2/3}$ c.c. saline;

β -suspension: Into 1 c.c. cholesterinised antigen is dropped 34 c.c. saline.

The antigens are prepared only in the quantities mentioned. If, for instance, 70 c.c. β -antigen is required, two lots, each of 35 c.c., are prepared.

The resulting suspensions should not be shaken, but are mixed by gently inverting the measuring cylinders.

The α - and β -suspensions are added to the serum dilutions according to the table, the α -suspension by a pipette, the β -suspension preferably by a Dreyer syringe.

Each sample must now be shaken thoroughly, covering the tube with the thumb, in order from right to left—*i.e.*, beginning with the tube containing the *highest* serum dilution. Dry the thumb on a cloth before passing from one tube to the next.

Place the stand in the dry-air incubator (38.5° C.) for twenty to twenty-two hours.

C. Reading of Results.

Place the stand on the agglutinoscope and read by help of the hand-lens. Compare the flocculation in the tubes with the Standard Flocculation Tube. Take the tube showing this degree of flocculation and find the corresponding strength in Sigma units, indicated on the label of the antigen bottle.

The figures given there have been checked with Professor Dreyer's Standard. If none of the tubes are showing exactly the standard flocculation, the value must be interpolated. In view of the possible presence of an inhibition zone, it is essential to examine at least the first five tubes in the series before pronouncing a serum negative.

Regarding the details in relation to standardisation, see Medical Research Council Report, No. 78.

Literature.

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Mörch: *The Lancet*, Vol. II, 1924, p. 58.

Bigger: *The Lancet*, Vol. II, 1924, p. 743.

Norel: *Zeitschr. f. Imm. & Exp. Therapie*, 1926, p. 475.

VERNES' REACTION.

1. *Method:*

The syphilimetric method.

2. *Principles:*

To produce flocculation in the blood serum and cerebro-spinal fluid by means of a chosen reagent and to carry out the investigations under such close co-operation between clinic and laboratory (instanced, up to the present, by more than 100,000 records at the Institut Prophylactique) that the presence or absence of flocculation indicates, not only the presence or absence of syphilis, but also, in the former case, the rises and falls occurring in the course of the infection.

3. *Advantages:*

(a) The elimination of the personal element, so that different workers testing the same sample in separate laboratories necessarily obtain the same results.

(b) *The measurement* — by means of an optical apparatus, the V.B.Y. photometer — of the variations produced by the fluctuations of the infection; the registration of these variations on a chart according to definite rules, and the use of the graphs in the conduct of treatment as a means of ensuring the successful prevention of syphilis, which consists in rendering the patient non-infectious not only for a time but for the remainder of his life. This is to his personal interest and is the only way of preventing hereditary syphilis.

4. *Temperature:*

Preliminary heating of the sera at 55° C.; performance of the test at room temperature of 19° to 21° C.; flocculation in the water-bath at 25° C.

5. *Reading of results:*

After four hours.

6. *Test-tubes used for each serum:*

Four.

7. *Number of pipettes per test:*

Nil.

8. *Other apparatus required:*

A rheometer; a mixing apparatus with propeller to regulate the size of the particles in the suspension, and a V.B.Y. photometer.

9. The technique employed at the Danish State Serum Institute for the examination of sera and cerebro-spinal fluids is that described in Part II of the Publications of the Paris Prophylactic Institute, pages 59 to 64 and 67 and 68.

Appendix.

METHODS DESCRIBED AT THE CONFERENCE BY
DR. A. SCALTRITTI

FLOCCULATION WITH THE USE OF OX OR PIG HEART LIPOIDS
PRECIPITATED BY CADMIUM CHLORIDE.

Procedure for obtaining the Lipoid.

The cardiac muscle of fresh ox or pig heart, freed from fat, is finely minced, and spread in thin layers on glass plates, which are placed in front of a revolving fan and dried as quickly as possible. When drying is complete, the muscle is pounded in a mortar and passed through a very fine sieve in order to obtain an impalpable powder.

The powder is treated with sulphuric ether for about three days, the ether being changed repeatedly until it remains colourless. This operation is carried out at room temperature.

The ether is now decanted and the powdered heart placed in the incubator at 37° C. for two hours, *i.e.*, long enough to dispel any remaining ether.

The dry powder, free of sulphuric ether, is then extracted in absolute alcohol in the proportion of one part of powder to six of alcohol for three days at room temperature, the mixture being shaken several times a day. At the end of that time, it is filtered through a double filter-paper. A transparent or very faintly yellowish liquid is thus obtained. It is from this solution that we precipitate the lipoids which, we believe, are required for the flocculation of the syphilitic sera. It is prepared once a week.

Method of Preparation.

As much of a super-saturated solution of cadmium chloride in absolute alcohol is prepared beforehand as will be needed for precipitation. As a rule, the proportion is 0.1 grammes of Cl_2Cd to every c.c. of absolute alcohol. The cadmium chloride is finely powdered in a glass mortar. It is then mixed with the alcohol and dissolved as completely as possible. The solution is placed in a small tube and spun for two to three minutes, until the alcohol becomes clear. It is then ready for use. 0.25 c.c. of this solution is placed in a small tube of the kind commonly used for Wassermann reactions. 2 c.c. of the alcoholic solution mentioned above is measured into another

tube. This solution is quickly poured on the first solution of Cl_2Cd and *vice versa*; the operation is repeated twice in order to mix the cadmium chloride as completely as possible with the lipoid solution. In this way a caseous precipitate is obtained. It is allowed to settle for half-an-hour and is then gently "centrifuged", allowing the centrifuge to make only a few revolutions.

The supernatant liquid is now carefully decanted and the precipitate thoroughly mixed with the small quantity of alcohol remaining at the bottom of the tube. The tube is then filled with 80 % alcohol, the precipitate becoming altered in appearance, and more nearly resembling snow. The tube is gently shaken to break up any lumps and is then set aside to allow the lipoid to settle. The alcohol is decanted and replaced by fresh alcohol, which is again decanted after being left to stand for a few moments. The tube is then half-filled with 80 % alcohol, and filled up with $8\frac{1}{2}/1000$ to $9/1000$ saline, care being taken not to shake the tube. If the lipoid should rise from the bottom of the tube, a slight centrifugal movement will make it settle again to the bottom.

Finally, the saline solution is decanted, leaving the lipoid almost free from cadmium chloride.

1 c.c. of physiological saline $8\frac{1}{2}/1000$ or $9/1000$ is then added and the mixture is emulsified by briskly shaking the tube, which must be well stoppered. To obtain a good emulsion ten minutes' shaking is needed. The emulsion is now ready. As many tubes as are required are prepared, remembering that for each reaction tube 0.05 c.c. is needed.

Technique of the Reaction.

Patient's serum inactivated at 56°C . for twenty to thirty minutes: 0.1 c.c.

Lipoid emulsion: 0.05 c.c.

The tubes are shaken briskly, preferably in a mechanical shaker, for a quarter of an hour.

By that time flocculi begin to appear in the case of strongly *positive* sera. In any case the tubes must be left at room temperature for eighteen to twenty hours, to allow time for the reaction to take place with weaker positive sera. If the test tubes are then examined with a lens in a suitable light, one can distinguish the tubes in which there is flocculation from those in which it is absent. This inspection should occupy but little time.

1 c.c. of $8\frac{1}{2}-9/1000$ saline is then added to each tube, which is gently shaken to obtain a good mixture.

Flocculation should now be more evident than at the first examination. The tubes are then briskly "centrifuged" for one or two minutes. The supernatant fluid is decanted, care being taken to prevent any lipoid passing over with it. Another c.c. of saline is added and the same operation is repeated.

Finally, 0.5 c.c. of saline is added, and the tube is gently rocked to and fro. It will then be seen that the flocculated lipoid no longer

forms an emulsion with the saline. There are degrees in this reaction from a thick, compact floccular mass to small loosened flakes floating in a transparent medium.

The non-flocculated lipoids readily form emulsions and become homogeneous. Reactions are classified as strongly, medium, or weakly positive, according to the intensity of flocculation.

In every case, a control tube containing 0.1 c.c. of saline and 0.05 c.c. of lipoid emulsion is put up and subjected to the same treatment as the tubes of the test proper.

PROCEDURE TO OBTAIN THE LIPOID USED IN THE BORDET-WASSERMANN REACTION.

Ox or pig heart (preferably the latter) is finely minced and dried with a revolving fan. It is then pounded and passed through a sieve. The powder is then treated with acetone and dried in an incubator at 37° C. for three or four hours until the acetone has evaporated.

The powder is soaked in absolute alcohol for three days at room temperature in the proportion of one part of powder to ten of alcohol. It is filtered through a double filter-paper. The result is an amber-coloured liquid from which the lipoids are precipitated by an alcoholic solution of Cl_2Cd .

(The lipid solution is prepared every four days.)

(For the preparation of the supersaturated alcoholic solution of Cl_2Cd , see the flocculation technique described above.)

1 ½ c.c. of lipid solution is poured quickly upon 0.25 c.c. of cadmium chloride solution and *vice versa*. The precipitate is allowed to settle. This is then washed in the same manner as the lipid in the flocculation reaction previously described.

The lipid having been obtained practically free from cadmium chloride, it is emulsified with 2 c.c. of saline by brisk shaking in a well-stoppered tube.

The bulk of fluid is brought up to 20 c.c. with saline at 8 ½/1000 and shaken for a further five minutes until a perfectly homogeneous emulsion is obtained. 0.2 c.c. of the emulsion is used in each reaction tube. This is much less than the anticomplementary dose.

As to the technique of the Bordet-Wassermann Reaction, we always employ that of Bordet-Ruelens.

Annex 3.

LIST OF THE INSTITUTIONS WHICH SENT BLOOD AND CEREBRO-SPINAL FLUID SPECIMENS FOR THE CONFERENCE.

Austria

- Professor Kerl, Vorstand der Klinik für Geschlechts- und Hautkrankheiten, Allgemeines Krankenhaus, Wien IX.
- Professor Wagner-Jauregg, Vorstand der Psychiatrischen Klinik, Allgemeines Krankenhaus, Wien IX.
- Professor Arzt, Vorstand der Klinik für Haut und Geschlechtskrankheiten, Allgemeines Krankenhaus, Wien IX.
- Professor Pappenheim, Vorstand der Nervenabteilung des Versorgungshauses, Wien XIII. Lainz.
- Herrn Docenten Brandweiner, Vorstand der dermatologischen Abteilung der Poliklinik, Wien IX. Mariannengasse.
- Herrn Professor Redlich, Primarius im Maria Theresienschlössel, Wien XIX, Hofzeile.
- Professor Oppenheim, Vorstand der dermatologischen Abteilung des Wilhelminenspitales, Wien XVI. Montleardgasse.
- Professor Porias, Vorstand der dermatologischen Abteilung des Rainerspitales, Wien XIII.
- Professor Mucha, Vorstand der Heilanstalt in Klosterneuburg bei Wien.
- Professor Kren, Vorstand der dermatologischen Abteilung des Krankenhauses der Stadt Wien, Wien XIII. Lainz.

Denmark (Copenhagen)

- Professor Bie, Blegdamshospitalet.
- Overlæge Bing, Kommunehospitalets II. Afd.
- Overlæge Bisgaard, St. Hans Hospital.
- Dr. H. Boas, Kommunens vederlagsfri Consultation.
- Overlæge Brun-Pedersen, Marinehospitalets Hudafd.
- Professor Ehlers, Kommunehospitalets IV. Afd.
- Professor Faber, Rigshospitalets Afd. B.
- Professor Gammeltoft, Rigshospitalets Fødeafd..
- Professor Hauch, Rigshospitalets Fødeafd.

Dr. Henningsen, Kommunens vederlagsfri Consultation.
Overlæge Jacobæus, Balders Hospital.
Overlæge Jersild, Rudolph Berghs Hospital.
Dr. A. Kissmeyer, Kommunens vederlagsfri Consultation.
Dr. H. Levy, Kommunens vederlagsfri Consultation.
Dr. Lomholt, Kommunens vederlagsfri Consultation.
Overlæge Meulengracht, Bispebjerg Hospital.
Overlæge L. Nielsen, Frederiksberg Hospitals Afd. C.
Dr. Pontoppidan, Kommunens vederlagsfri Consultation.
Professor Rasch, Rigshospitalet.
Overlæge Reyn, Finsens medicinske Lysinstitut.
Overlæge Thalbitzer, St. Hans Hospital.
Overlæge Tobiesen, Øresundshospitalet.
Overlæge Vogelius, St. Johannes Stiftelse.
Professor Wimmer, Kommunehospitalets VI. Afd.
Overlæge Würtzen, Øresundshospitalet.

France

Dr. Vernes, Directeur de l'Institut Prophylactique, Paris.
Professor Desbains, Hôpital de Versailles.

Germany

Professor Otto (Berlin), and his colleagues from:

Rudolf Virchow-Krankenhaus (Hautkliniken und Infektions-
krankenabteilung), Berlin.

Charité-Krankenhaus (Hautklinik und Poliklinik für Krebskranke),
Berlin.

Staat-Krankenhaus Moabit (Innere Abteilung), Berlin.

Heilstätten Wittenau in Berlin-Wittenau; und

Viktoria-Luise-Kinderheilstätte, Hohenlychen Kreis Templin,
Berlin.

Dr. Jacobsthal, Allgemeines Krankenhaus, Hamburg.

Professor Nocht, Institut für Schiffs- und Tropenkrankheiten,
Hamburg.

Great Britain

Colonel L. W. Harrison and colleagues of the Venereal Diseases
Treatment Centre, St. Thomas's Hospital, London.

APPENDIX

Comparison of the Kahn with the Bordet-Wassermann Method (No. 1 Medical Research Council Report Series No. 14) in Tests of Cerebro-Spinal Fluids, carried out by Dr. Kahn and Dr. Wyler in London after the Conference, July 10th to 25th, 1928.

(The Kahn Test was carried out by Professor Kahn in the Pathological Laboratory of the Venereal Diseases Treatment Centre, St. Thomas's Hospital, and the B.-W. Test was carried out by Dr. Wyler in the Ministry of Health's Special Laboratory, Medical School, St. Thomas's Hospital. Five hundred and fifty-five cerebro-spinal fluids were tested.)

Table A.

SUMMARY OF 317 TESTS IN WHICH BOTH WORKERS REPORTED THE SPECIMENS AS "CLEAR".

Diagnosis		Totals	Kahn					Wyler (B.-W.)					
			Positive			Doubt-ful.	Neg.	Positive			Doubt-ful.	Neg.	
			++	+	Total ++ & +	±	—	++	+	Total ++ & +	±	—	
<i>Syphilis :</i>													
I	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	2	—	—	—	—	2	—	—	—	—	2	
II	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	3	—	—	—	1	2	—	—	—	—	3	
III	U.T.	2	—	—	—	—	2	—	—	—	1	1	
	T.	12	3	—	3	—	9	1	1	2	1	9	
Latent	U.T.	4	1	—	1	—	3	1	—	1	—	3	
	T.	12	1	—	1	1	10	1	—	1	3	8	
Congenital	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	10	4	—	4	—	6	3	1	4	—	6	
Tabes	U.T.	12	6	—	6	—	6	4	1	5	3	4	
	T.	73	30	3	33	6	34	21	8	29	19	25	
G.P.I.	U.T.	66	35	—	35	—	31	33	1	34	6	26	
	T.	51	32	4	36	4	11	26	8	34	9	8	
Tabo- paresis	U.T.	1	1	—	1	—	—	—	—	—	1	—	
	T.	2	1	1	2	—	—	2	—	2	—	—	
Other forms	U.T.	6	2	—	2	—	4	1	1	2	—	4	
	T.	36	13	—	13	3	20	6	9	15	3	18	
Totals		U.T.	91	45	—	45	—	46	39	3	42	11	38
		T.	201	84	8	92	15	94	60	27	87	35	79
Total Syphilis		(U.T. & T.)	292	129	8	137	15	140	99	30	129	46	117
<i>Controls</i>			25	—	—	—	21	24	—	—	—	24	21

¹ U.T. — untreated. T. — treated.

² No. 377, Mental, doubtful signs of D.p.

³ No. 30, Facial Palsy, non-syphilitic; No. 591, Dementia præcox; No. 205, Mental, physical signs only suggestive of D.p., and No. 277, Mental, doubtful signs of D.p.

Table B.

SUMMARY OF 238 TESTS IN WHICH ONE OR BOTH WORKERS REPORTED THE SPECIMENS AS "CLOUDY", AS CONTAMINATED, OR AS CONTAINING "SOME BLOOD".

Diagnosis		Totals	Kahn					Wyler (B.-W.)					
			Positive			Doubt-ful.	Neg.	Positive			Doubt-ful.	Neg.	
			++	+	Total ++ & +	±	—	++	+	Total ++ & +	±	—	
<i>Syphilis :</i>													
I	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	1	—	—	—	—	1	—	—	—	—	1	
II	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	3	—	—	—	—	3	—	—	—	—	3	
III	U.T.	2	1	—	1	—	1	1	—	1	—	1	
	T.	13	2	—	2	—	11	1	1	2	—	11	
Latent	U.T.	—	—	—	—	—	—	—	—	—	—	—	
	T.	6	—	—	—	—	6	—	—	—	—	6	
Congenital	U.T.	1	—	—	—	—	1	—	—	—	—	1	
	T.	2	1	—	1	—	1	1	—	1	1	—	
Tabes	U.T.	16	5	—	5	—	11	7	—	7	2	7	
	T.	48	19	4	23	7	18	14	13	27	7	14	
Neuro-	G.P.I.	U.T.	25	14	2	16	—	9	13	3	16	2	7
	T.	59	38	3	41	4	14	31	14	45	8	6	
Tabo- paresis	U.T.	2	1	1	2	—	—	2	—	2	—	—	
	T.	4	3	—	3	—	1	2	—	2	1	1	
Other forms	U.T.	9	4	—	4	—	5	2	1	3	1	5	
	T.	25	6	—	6	1	18	4	1	5	5	15	
Totals	U.T.	55	25	3	28	—	27	25	4	29	5	21	
	T.	161	69	7	76	12	73	53	29	82	22	57	
Total Syphilis		(U.T. & T.)	216	94	10	104	12	100	78	33	111	27	78
<i>Controls</i>			22 ¹	—	—	—	22 ¹	—	—	—	—	—	22 ¹

¹ Includes 2 cases "Not yet diagnosed".

Table C.

SUMMARY OF THE 555 TESTS SHOWN IN TABLES A AND B.

Diagnosis		Totals	Kahn					Wyler (B.-W.)						
			Positive			Doubt-ful.	Neg.	Positive			Doubt-ful.	Neg.		
			++	+	Total ++ & +	±	—	++	+	Total ++ & +	±	—		
<i>Syphilis :</i>														
I	U.T.	—	—	—	—	—	—	—	—	—	—	—		
	T.	3	—	—	—	—	3	—	—	—	—	3		
II	U.T.	—	—	—	—	—	—	—	—	—	—	—		
	T.	6	—	—	—	1	5	—	—	—	—	6		
III	U.T.	4	1	—	1	—	3	1	—	1	1	2		
	T.	25	5	—	5	—	20	2	2	4	1	20		
Latent	U.T.	4	1	—	1	—	3	1	—	1	—	3		
	T.	18	1	—	1	1	16	1	—	1	3	14		
Congenital	U.T.	1	—	—	—	—	1	—	—	—	—	1		
	T.	12	5	—	5	—	7	4	1	5	1	6		
Neuro-	Tabes	U.T.	28	11	—	11	—	17	11	1	12	5	11	
		T.	121	49	7	56	13	52	35	21	56	26	39	
	G.P.I.	U.T.	91	49	2	51	—	40	46	4	50	8	33	
		T.	110	70	7	77	8	25	57	22	79	17	14	
	Tabo- paresis	U.T.	3	2	1	3	—	2	—	2	1	—	—	
		T.	6	4	1	5	—	1	4	—	4	1	1	
	Other forms	U.T.	15	6	—	6	—	9	3	2	5	1	9	
		T.	61	19	—	19	4	38	10	10	20	8	33	
	Totals		U.T.	146	70	3	73	—	73	64	7	71	16	59
			T.	362	153	15	168	27	167	113	56	169	57	136
Total Syphilis		(U.T. & T.)	508	223	18	241	27	240	177	63	240	73	195	
<i>Controls</i>			47 ¹	—	—	—	1 ²	46 ¹	—	—	—	4 ³	43 ¹	

¹ Includes 2 cases "Not yet diagnosed".

² No. 377, Mental, doubtful signs of D. p.

³ No. 30 Facial Palsy, Non-syphilitic; No. 591 Dementia præcox; No. 205, Mental, physical signs only suggestive of D. p. and No. 277, Mental, doubtful signs of D. p.

Details of Results included in Table A i

(In the cases marked * the diagnosis, or the classification into "treated" of the results to the institution)

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis
		C. S. F.	C. S. F.	
18.VII.28	251	++	+	D.p. Untreated.
20.VII.28	330	++	+	Tabes. "
13.VII.28	561	++	+	Other forms. Neuro-S. "
11.VII.28	26	++	+	D.p. Treated.
11.VII.28	48	++	+	Autres formes. Neuro-S. "
11.VII.28	61	++	+	Cong. S. "
12.VII.28	109	++	+	Tabes. "
18.VII.28	232	++	+	Other forms. Neuro-S. "
18.VII.28	254	++	+	D.p. "
19.VII.28	279	++	+	S. III. "
19.VII.28	291	++	+	Other forms. Neuro-S. "
20.VII.28	314	++	+	D.p. "
20.VII.28	337	++	+	Tabes. "
20.VII.28	345	++	+	D.p. "
25.VII.28	424	++	+	D.p. "
11.VII.28	529	++	+	Other forms. Neuro-S. "
12.VII.28	539	++	+	Tabes. "
12.VII.28	554	++	+	Other forms. Neuro-S. "
13.VII.28	571	++	+	D.p. "
24.VII.28	659	++	+	Other forms. Neuro-S. "
11.VII.28	55	+	++	Tabo-paresis. "
13.VII.28	180	+	++	Tabes. "
10.VII.28	458	+	++	Tabes. "
12.VII.28	542	+	++	D.p. "
18.VII.28	223	+	±	D.p. "
18.VII.28	231	+	±	D.p. "
20.VII.28	299	+	±	Tabes. "
13.VII.28	179	±	+	Tabes. "
18.VII.28	246	±	+	Tabes. "
19.VII.28	285	±	+	Other forms. Neuro-S. "

which the Kahn and B.-W. Results differed.

"untreated", has been changed as a result of further inquiry after submission which provided the specimens.)

Remarks	Cell Count. (Wyer)	Globulin ²			B.-W. of serum taken same time as fluid ³	
		Kahn	Wyer ¹		Date tested	Result
			N	P		
		++	+	+	11.VII.28	++
		++	—	+	7.VII.28	++
		++	+	+	13.VII.28	++
	5	+	—		18.VII.28	±
	2	++	—		18.VII.28	±
	0	±	—	—	18.VII.28	++
		++	+	+	20.VII.28	++
		±	—	+	5.VII.28	±
Malaria and Tryparsamide.		++	+	+	11.VII.28	A.C.
	1	+	D	—	7.VII.28	++
		+	—	+	7.VII.28	±
Malaria Jan. 1928.		++	+	+	7.VII.28	++
		++	—	+	7.VII.28	++
Malaria April 1926.		++	D	+	7.VII.28	—
Malaria 1927.		++	+	+	7.VII.28	++
		++	No test		13.VII.28	—
		++	—	D	13.VII.28	+
		++	+	+	13.VII.28	±
Malaria Dec. 1927, followed by Hg., Iod., Sulfarsenol and Tryparsamide.		++	+	+	13.VII.28	++
		+	—	+	26.VII.28	++
	13	++	+	+	18.VII.28	++
		++	D	+	2.VII.28	++
		+	—	+	11.VII.28	++
Malaria Aug. 1927.		++	—	+	13.VII.28	++
Malaria Tr. (Also Tabetic)	4	++	+	+	5.VII.28	++
	1	+	D	D	5.VII.28	++
		+	—	D	7.VII.28	++
Optic atrophy.	3	±	—	D	2.VII.28	—
	0	±	D	D	5.VII.28	±
Paresis Ext. rectus. Agyll-Robertson pupils.		++	+	+	7.VII.28	++

¹ N = Nonne; P = Pandey; D = doubtful.

² Carried out by Dr. T. E. Osmond by No. 1 Method.

Explanation of abbreviations:

S.C. = slow lysis of serum control.

A.C. = anticomplementary.

— N.C. = negative not clear.

³ It is to be noted that Professor Kahn graded his globulin test results: "++", "+", "±", and "—" whilst Dr. Wyler graded his results: "++", "D", and "—".

Details of Results included in Table A in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F	C. S. F.		
24.VII.28	397	±	+	Tabes.	Treated.
12.VII.28	543	±	+	D.p.	"
19.VII.28	635	±	+	Other forms. Neuro-S.	"
24.VII.28	377	±	—	*Control.	
17.VII.28	194	±	—	S. II.	"
11.VII.28	31	—	±	S. III.	Untreated.
17.VII.28	200	—	±	D.p.	"
17.VII.28	205	—	±	*Control.	
19.VII.28	277	—	±	*Control.	
20.VII.28	304	—	±	Tabes.	"
20.VII.28	306	—	±	Tabes.	"
24.VII.28	379	—	±	D.p.	"
24.VII.28	386	—	±	D.p.	"
24.VII.28	387	—	±	D.p.	"
11.VII.28	47	—	±	Tabes.	Treated.
11.VII.28	60	—	±	Tabes.	"
11.VII.28	64	—	±	D.p.	"
12.VII.28	93	—	±	*D.p.	Untreated.
12.VII.28	115	—	±	Tabes.	Treated.
13.VII.28	159	—	±	Other forms. Neuro-S.	"
19.VII.28	280	—	±	Latent S.	"
19.VII.28	292	—	±	S. III.	"
19.VII.28	293	—	±	Tabes.	"
19.VII.28	295	—	±	Tabes.	"
19.VII.28	296	—	±	Tabes.	"
24.VII.28	375	—	±	D.p.	"
10.VII.28	469	—	±	Tabes.	"
11.VII.28	522	—	±	Tabes.	"
13.VII.28	565	—	±	Tabes.	"

he Kahn and B.-W. Results differed (Continued).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
		+	—	+	11.VII.28	++
Malaria Feb. 1928.		+	—	—	30.VII.28	++
Progressive muscular atrophy.		++	+	+	23.VII.28	++
Mental (doubtful signs of D.p.).		—	—	—	9.VII.28	—
	0	—	—	D	2.VII.28	—
? Gumma of Lung. Previous test of C.S.F. negative.	0	±	—		18.VII.28	—
Juvenile D.p.	0	±	—	—	2.VII.28	—
Mental ; physical signs only suggestive of D.p.	1	±	—		2.VII.28	—
Mental ; doubtful signs of D.p.		±	—	—	7.VII.28	—
		±	—	—	7.VII.28	++
		±	—	—	7.VII.28	++
Definite signs of D.p.		+	—	+	9.VII.28	—
Definite signs of D.p.		—	—	—	9.VII.28	—
Unequivocal signs of syphilis of central nervous system.		—	—	—	9.VII.28	—
	2	+	D	—	18.VII.28	±
	0	+	—	D	18.VII.28	++
Malaria Jan. 1927.	19	++	D	D	18.VII.28	+
Unequivocal signs of syphilis of central nervous system.		++	—	D	18.VII.28	++
	2	+	—	—	20.VII.28	A.C.
Paralysis Right external rectus.		++	No test		20.VII.28	++
	0	++	—		7.VII.28	++
? Early tabes, but previous Lange, 0000000000 and cells, 1.		—	—	—	7.VII.28	++
		+	—	D	7.VII.28	++
		+	—	—	7.VII.28	++
		—	—	—	7.VII.28	±
One course Tryparsamide.		+	D	+	9.VII.28	++
		±	—	—	11.VII.28	++
Optic atrophy.		+	+	+	13.VII.28	±±
		+	—	—	13.VII.28.	++

Details of Results included in Table A in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C S. F.		
19.VII.28	625	—	±	Latent S.	Treated.
19.VII.28	637	—	±	D.p.	”
20.VII.28	339	++	±	*Tabo-paresis.	Untreated.
11.VII.28	499	++	±	D.p.	”
13.VII.28	564	++	±	Tabes.	”
10.VII.28	8	++	±	Tabes.	Treated.
11.VII.28	56	++	±	Tabes.	”
12.VII.28	111	++	±	Tabes.	”
12.VII.28	116	++	±	Other forms. Neuro-S.	”
18.VII.28	245	++	±	Tabes.	”
25.VII.28	402	++	±	Tabes.	”
25.VII.28	429	++	±	D.p.	”
17.VII.28	587	++	±	?Tabes.	”
19.VII.28	627	++	±	Tabes.	”
20.VII.28	648	++	±	D.p.	”
25.VII.28	422	±	++	D.p.	”
19.VII.28	634	±	++	*Tabes.	”
12.VII.28	110	++	—	Tabes.	”
20.VII.28	308	++	—	Tabes.	”
24.VII.28	359	++	—	S. III.	”
13.VII.28	181	—	+	Other forms. Neuro-S.	”
13.VII.28	184	—	+	?Tabes.	”
18.VII.28	238	—	+	Tabes.	”

the Kahn and B.-W. Results differed (*Concluded*).

Remarks	Cell Count. (Wyller)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyller		Date tested	Result
			N.	P.		
Malaria.		+	D	—	20.VII.28	++
Perforation septum nasi. Giddiness, nystagmus, Knee Jerks +.		+	D	D	No serum.	
		++	D	+	7.VII.28	±
		+	—	+	13.VII.28	++
		+	D	D	13.VII.28	++
	2	±	—		No serum.	
		++	+	+	18.VII.28	±
		+	D	D	20.VII.28	++ S.C.
R. Ptosis, L. Hemiplegia.	30	+	D	D	20.VII.28	++
	1	+	—	—	5.VII.28	++
		+	D	+	11.VII.28	±
Malaria.		++	—	+	11.VII.28	++
Optic atrophy.		++	+	+	16.VII.28	++
W.R. of blood negative prior to this.		±	—	D	No serum.	
Malaria.		++	+	+	23.VII.28	±
		++	+	+	11.VII.28	++
Paralysis r. hand 1927. Pupil light reflex nil. Knee Jerk nil.		++	—	—	20.VII.28	++
		+	—	—	20.VII.28	—
		+	No test		No serum.	
		++	—	+	7.VII.28	++
S. meningitis. Previously diagnosed D.p.	2	++	+	+	2.VII.28	++
Optic atrophy.		+	—	—	2.VII.28	++
	0	+	—	D	5.VII.28	—

Details of Results included in Table B in

(In the cases marked * the diagnosis, or the classification into "treated" or of the results to the institutions

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
18.VII.28	252	++ cloudy	+ cloudy	D.p.	Untreated.
25.VII.28	430	++ cloudy	+	D.p.	"
24.VII.28	655	++ cloudy	+ cloudy	Other forms. Neuro-S.	"
10.VII.28	14	++ cloudy	+ cloudy	D.p.	Treated.
12.VII.28	76	++ cloudy	+ cloudy	S. III.	"
13.VII.28	151	++ cloudy	+ cloudy	D.p.	"
13.VII.28	161	++	+ cloudy	Tabes.	"
13.VII.28	187	++	+ cloudy	Tabes.	"
13.VII.28	188	++	+ cloudy	Tabes.	"
18.VII.28	211	++ cloudy	+ cloudy	Tabes.	"
19.VII.28	270	++ cloudy	+ cloudy	Tabes.	"
20.VII.28	342	++ contains blood	+ some blood	D.p.	"
20.VII.28	343	++ cloudy	+	D.p.	"
20.VII.28	346	++ cloudy	+ cloudy	D.p.	"
24.VII.28	368	++ cloudy	+ cloudy	D.p.	"
25.VII.28	413	++ cloudy	+ cloudy	D.p.	"
25.VII.28	414	++ cloudy	+ cloudy	D.p.	"
25.VII.28	416	++ cloudy	+ cloudy	D.p.	"
10.VII.28	462	++ cloudy	+ cloudy	D.p.	"
11.VII.28	513	++sl. cloudy	+ cloudy	D.p.	"
11.VII.28	515	++sl. cloudy	+ cloudy	Tabes.	"
12.VII.28	555	++	+ cloudy	Other forms. Neuro-S.	"
12.VII.28	89	+ cloudy	++ cloudy	D.p.	Untreated.
11.VII.28	537	+ cloudy	++ cloudy	Tabo-paresis.	"
10.VII.28	40	+	++ cloudy	D.p.	Treated.
12.VII.28	94	+ cloudy	++ cloudy	D.p.	"
24.VII.28	372	+ cloudy	++ cloudy	D.p.	"
24.VII.28	394	+ cloudy	++ cloudy	? Tabes.	"
12.VII.28	557	+	++ cloudy	Tabes.	"
12.VII.28	559	+	++ cloudy	? Tabes.	"

which the Kahn and B.-W. Results differed.

"untreated", has been changed as a result of further inquiry after submission which provided the specimens.)

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
Malaria April 1927.	0 2	++	+		11.VII.28	++
		++	D	+	11.VII.28	++
		+	No test		26.VII.28	++
		++	—		18.VII.28	++
		++	—		18.VII.28	++
		++	—	+	20.VII.28	±
		++	No test		20.VII.28	++
		+	,,		2.VII.28	++
		++	,,		2.VII.28	++
		+	,,		5.VII.28	++
Malaria Sept. 1926.		++	D	+	5.VII.28	±
		++	No test		7.VII.28	++
Malaria Feb. 1928.		++	,,		7.VII.28	++
Malaria June 1926.		++	,,		7.VII.28	++
Malaria Oct. 1927.		++	,,		7.VII.28	++
Malaria and Tryparsamide.		++	,,		11.VII.28	++
Malaria and Tryparsamide		++	,,		11.VII.28	++
Malaria and Tryparsamide.		++	,,		11.VII.28	++
		++	+	+	11.VII.28	++
Malaria 1924.		++	No test		13.VII.28	±
Malaria 1928.		++	,,		13.VII.28	++
		++	,,		13.VII.28	++
		++	+		18.VII.28	++
		++	No test		13.VII.28	++
		++	+		18.VII.28	++
Malaria June 1927.	6	++	+		18.VII.28	++
		++	+		18.VII.28	++
Malaria Oct. and Nov. 1926.		+	No test		9.VII.28	++
		+	,,		9.VII.28	—
		++	+	+	No serum.	
		++	No test		13.VII.28	++

Details of Results included in Table B in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
19.VII.28	262	+cloudy, contaminated, greenish	± cloudy, some blood	D.p.	Untreated.
24.VII.28	395	+ cloudy	± cloudy	Tabes.	Treated.
12.VII.28	108	± cloudy	+ cloudy	Tabes.	"
13.VII.28	570	±	+ cloudy	D.p.	"
20.VII.28	301	± cloudy	— cloudy, infected	D.p.	"
10.VII.28	464	± cloudy, contaminated.	— some blood, inactivated.	Tabes.	"
10.VII.28	465	± cloudy, contaminated.	— cloudy	Tabes.	"
12.VII.28	106	— cloudy	± cloudy	Tabes.	Untreated.
13.VII.28	137	—	± cloudy	D.p.	"
19.VII.28	288	— cloudy	± cloudy	Tabes.	"
11.VII.28	54	— sl.cloudy	± cloudy	Tabes.	Treated.
12.VII.28	97	— cloudy	± cloudy	Congl. S.	"
13.VII.28	136	— cloudy, bloody	± hæmoglobin	Other forms. Neuro-S.	"
24.VII.28	369	— cloudy	± cloudy	D.p.	"
25.VII.28	403	— cloudy	±	D.p.	"
25.VII.28	449	— cloudy	± cloudy	D.p.	"
13.VII.28	577	— cloudy	± cloudy	D.p.	"
17.VII.28	611	—	± cloudy	Other forms. Neuro-S.	"
24.VII.28	656	— cloudy	±	Other forms. Neuro-S.	"

Kahn and B.-W. Results differed (Continued).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
		++	„		11.VII.28	++
		±	„		9.VII.28	-
Argyll-Robertson pupils. Knee Jerks absent.		++	D	+	20.VII.28	+ S.C.
Malaria Aug. 1926. Typical. G.P.I. Dull, irritable, untidy.		+	No test		(1.VII.28 (see No. 364.)	++)
		++	„		7.VII.28	-
Knee and Achilles Jerks absent.		++	„		11.VII.28	-
Also hemiparesis. K.J's and A.J's absent.		+	-	D	11.VII.28	±
Clinically typical.		++	+	+	20.VII.28	++S.C.
Definite signs of D.p.		+	-	-	20.VII.28	-
C.S.F. ++ on 24.IV.20.		+	No test		7.VII.28	-N.C.
K.J's absent. Malarial Tr. followed by Silv.-Salv. and Sulfarsenol.	0	±	-		18.VII.28	+
Intra-cisternal injections, though no clinical cerebro-spinal symptoms.	0	±	-		18.VII.28	±
Ataxia, Romberg A.R. pupils ; K.J. ++. No sensory changes.		++	No test		20.VII.28	++
Malaria May 1925. At that time C.S.F. positive W.R. Cells ++, Globulin ++.		++	„		7.VII.28	++
Malaria and Tryparsamide.		+	+	+	11.VII.28	±
Malaria April 1926.		++	No test		11.VII.28	-
Malaria and Salvarsanised serum.		++	„		16.VII.28	++
Paralysis 3rd cranial.		-	„		20.VII.28	±
R. pupils, K.J. +; W.R. of C.S.F. 1926, ++, 1927, ±.		+	-	D	26.VII.28	++

Details of Results included in Table B in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
24.VII.28	654	++ bloody	± some blood	Other forms. Neuro-S.	Treated.
11.VII.28	39	++ sl. cloudy	± cloudy	D.p.	"
11.VII.28	44	++ sl. cloudy	± cloudy	D.p.	"
11.VII.28	53	++ sl. cloudy	± cloudy	Tabes.	"
18.VII.28	226	++	± cloudy	Other forms. Neuro-S.	"
24.VII.28	396	++	± some blood	Tabo-paresis.	"
13.VII.28	562	++	± cloudy	Tabes.	"
13.VII.28	566	++ cloudy, bloody	± some hæmo-globin	Other forms. Neuro-S.	"
13.VII.28	569	++	± cloudy	Tabes.	"
11.VII.28	43	± sl. cloudy	++ cloudy	D.p.	"
13.VII.28	182	±	++ cloudy	Other forms. Neuro-S.	"
24.VII.28	390	±	++ cloudy	Tabes.	"
18.VII.28	243	— cloudy heavily contaminated,	++ cloudy	Tabes.	Untreated
10.VII.28	496	— cloudy, contaminated.	++ cloudy	Tabes.	"
12.VII.28	98	— cloudy	++ cloudy	D.p.	Treated.
13.VII.28	144	—	++ cloudy	*Tabes.	"
18.VII.28	250	— cloudy	+ cloudy	D.p.	Untreated.
12.VII.28	70	—	+ cloudy	D.p.	Treated.
13.VII.28	134	— cloudy	+ cloudy	Tabes.	"
13.VII.28	183	— cloudy	+ some blood	D.p.	"
12.VII.28	541	— sl. cloudy	+ cloudy	D.p.	"

the Kahn and B.-W. Results differed (Continued).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
A.R. pupils, right side Hemiplegia. K.J's ++. Right Babinski.		++	No test		26.VII.28	—
Malaria Nov. 1927.	0	++	—		18.VII.28	++
Malaria June 1927.	1	++	D		18.VII.28	++
	0	++	—		18.VII.28	++
Cerebral endarteritis; Aneurism Aorta; Choroiditis.	0	+	—	D	5.VII.28	—
		++	No test		11.VII.28	++
K.J's absent. Ataxic gait.		++	,,		13.VII.28	++
Cerebral.		++	,,		13.VII.28	++
Charcot's disorganisation of rt. knee joint. K.J. rt. leg absent.		+	,,		13.VII.28	±±
Malaria Oct. 1927.	2	++	+		18.VII.28	++
Diplopia. Headache.		+	No test		2.VII.28	++
K.J's absent. Ataxia. Rombergism.		+	,,		9.VII.28	++
Optic atrophy. Ataxia. Romberg.		++	—		5.VII.28	++
A.R. pupils. Ptosis. Nystagmus.		++	No test		No serum.	
Typical.	3	++	+	+	18.VII.28	++
		++	D	+	20.VII.28	++
Stationary.		+	—		11.VII.28	++
Malaria Dec. 1925.	0	++	—		18.VII.28	—
Ataxia. K.J's absent. Incontinence urine.		++	+		20.VII.28	±
Probably tabo-paresis.		+	No test		2.VII.28	++
Malaria Aug. 1925.		++	,,		13.VII.28	++

Details of Results included in Table B in which

Date of Test	Research No.	Kahn (Dr. Kahn)	B.-W. (Dr. Wyler)	Diagnosis	
		C. S. F.	C. S. F.		
17.VII.28	592	—	+ cloudy	Tabes.	Treated.
17.VII.28	604	—	+ cloudy	Tabes.	„
19.VII.28	629	— cloudy, contami- nated, greenish.	+ cloudy	Tabes.	„
24.VII.28	653	— cloudy	+ cloudy	Tabes.	„

the Kahn and B.-W. Results differed (*Concluded*).

Remarks	Cell Count. (Wyler)	Globulin			B.-W. of serum taken same time as fluid	
		Kahn	Wyler		Date tested	Result
			N.	P.		
Aneurism.		+				
Lange, luetic curve 1924 and 1925 ;		±				
now almost neg.						
W.R. of C.S.F. pos. since 1922.		++	-	-	20.VII.28	-
Optic atrophy.		+			26.VII.28	±

**COMMENTS ON THESE, TESTS PRESENTED BY
COLONEL L. W. HARRISON, Dr. KAHN AND
Dr. WYLER**

REPORT BY COLONEL L. W. HARRISON.

As it had been found impracticable in the case of the cerebro-spinal fluid to obtain a sufficient number of specimens in sufficient amounts to afford a reliable comparison with all the different tests employed at Copenhagen, it was decided after the Conference to take the opportunity of Dr. Kahn's presence in London for a few weeks to carry out a comparison of the Kahn with the Bordet-Wassermann test (Harrison-Wyler method).

By the courtesy of the British Ministry of Health, the services of Dr. Wyler and the special laboratory at St. Thomas's Hospital were placed at the disposal of the League of Nations Health Organisation for the purpose; and, by that of the Treasurer and Almoner of St. Thomas's Hospital, laboratory facilities were afforded to M. Kahn in the V.D. Department of that institution.

At the request of Colonel Harrison, 555 specimens of cerebro-spinal fluid were collected by the chief physicians of the clinics indicated in the list at the end of this report, to whom the League of Nations Health Organisation would gratefully acknowledge its indebtedness for valuable assistance in this important investigation.

Most of the specimens of cerebro-spinal fluid were accompanied by specimens of blood serum from the same patients. It was found impracticable in the time available for either Dr. Kahn or Dr. Wyler to undertake the testing of these sera, and this work was carried out by Dr. T. E. Osmond, Pathologist to the V.D. Department of St. Thomas's Hospital, for whose help, given at a time of great pressure of other work, the League of Nations Health Organisation desires to express its thanks. Dr. Osmond employed No. 1 Method of the Medical Research Council Special Report Series No. 14, which is the same as that used by Dr. Wyler at the Copenhagen Conference.

It was not found practicable to carry out cell counts, as was hoped, on any but a small proportion of the specimens

of cerebro-spinal fluid, nor could any specimens be tested by a colloidal method such as the Lange, mastix or benzoin.

Owing possibly to the hot weather and the fact that most of the specimens had to travel by post, about half were found at the time of testing to be cloudy. In addition, a certain number were rejected as too heavily contaminated. It was felt that cloudiness of a specimen might affect the test or its reading, and for this reason the results have been analysed in two sections—cloudy and clear respectively—in Tables A and B. An analysis of the combined results is shown in Table C.

Analysis of the 317 tests of clear fluid shown in Table A.

Agreements					
		Untreated	Treated	Total	
++ or +		42	76	118	
±		0	6	6	
—		38	75	113	
		80	157	237	

Disagreements					
	Kahn	B.-W.	Untreated	Treated	Total
(a)	++ or +	±	3	13	16
(b)	±	++ or +	0	8	8
(c)	±	—	0	1	1
(d)	—	±	8	16	24
(e)	++ or +	—	0	3	3
(f)	—	++ or +	0	3	3

Thus there was substantial agreement between the two tests in approximately 75 per cent. There were no important disagreements or non-specific reactions in control cases.

The disagreements show broadly that the Kahn test gave stronger reactions with 20 specimens [lines (a), (c), (e)] and the B.-W. stronger reactions with 35 [lines (b), (d), (f)]. If the 25 disagreements between ± and — [lines (c) and (d)] are ignored, the Kahn test gave 19 stronger reactions (++ or + against ± or —) than the B.-W. [lines (a), (e)], and the B.-W. gave 11 stronger reactions than the Kahn [lines (b), (f)].

In treated cases of syphilis, however, a ± reaction is usually regarded as important. If, in such cases, it is

accepted as evidence of persistent infection, the Kahn test gave no sign of such persistence (by a ++, + or ± reaction) in 19 treated cases where the B.-W. test gave one or other of these reactions [lines (d) and (f)] and the reverse occurred in 4 [lines (c) and (e)].

Analysis of the 238 tests of cloudy fluid shown in Table B.

Agreements						
			Untreated	Treated	Total	
	++	or +	26	67	93	
		±	0	4	4	
		—	21	54	75	
			47	125	172	

Disagreements							
	Kahn		B.-W.		Untreated	Treated	Total
(a)	++	or +		±	2	9	11
(b)		±	++	or +	0	5	5
(c)		±		—	0	3	3
(d)		—		±	3	9	12
(e)	++	or +		—	0	0	0
(f)		—	++	or +	3	10	13

Thus, in this series of cloudy specimens, there was substantial agreement in approximately 72 per cent.

There were no disagreements or non-specific reactions in "Controls".

The disagreements show broadly that the Kahn test gave stronger reactions than the B.-W. in 14 cases and the B.-W. stronger reactions than the Kahn in 30.

If the 15 disagreements between ± and — [lines (c) and (d)] are ignored, the Kahn test gave 11 stronger reactions [lines (a) and (e)] than the B.-W. and the B.-W. test gave 18 stronger reactions [lines (b) and (f)] than the Kahn.

Considering only the treated cases and accepting a ± reaction as significant of persisting infection, the Kahn test gave no sign of such persistence (by a ++, + or ± reaction) in 19 treated cases where the B.-W. test gave one of these reactions [see lines (d) and (f)] and the reverse occurred in 3 [see lines (c) and (e)].

Comparing the two series of results in clear and cloudy specimens respectively, it will be seen that there is a quite definite difference. Thus, in the specimens from syphilitic cases, the percentages of reactions were as follows:

Cases of syphilis, treated and untreated.

	<i>Clear specimens</i>		<i>Cloudy specimens</i>	
	Kahn	B.-W.	Kahn	B.-W.
++ or +	46.9	44.1	48.1	51.3
±	5.1	15.7	5.5	12.5
—	47.9	40.0	46.3	36.1

If the treated cases of syphilis only are considered and in these the ± reactions are added to the ± and ++, on the grounds of a ± reaction being significant in a treated case, the percentages of reactions were as follows.

Cases of treated syphilis.

	<i>Clear specimens</i>		<i>Cloudy specimens</i>	
	Kahn	B.-W.	Kahn	B.-W.
++, + or ±	53.2	60.6	54.6	64.5
—	46.7	39.3	45.3	35.4

In both cases, a higher percentage of positive reactions was obtained in the cloudy series, but the preponderance of such reactions was more pronounced in the case of the B.-W. test than in that of the Kahn.

A possible explanation is that the specimens in the cloudy series happened to be inherently more syphilitic, but their physical condition of cloudiness interfered with the reading of the Kahn reaction.

COMMENTS OF DR. R. L. KAHN.

(University of Michigan Hospital, Ann Arbor, Michigan, U.S.A.)

1. I read Colonel Harrison's report of the spinal fluid comparison with great care, and I am greatly indebted to Colonel Harrison for giving a considerable amount of time and labour in making this series of spinal fluid studies possible and in preparing this very clear report.

2. There is one point about which Colonel Harrison and I disagree in principle; namely, as to the value of a plus-minus (\pm) spinal fluid reaction in the diagnosis and treatment of neurosyphilis. In the examination of a serum for the detection of syphilis, a plus-minus (\pm) reaction is of some value. A clinician may not suspect syphilis and a reaction of this type may give him a clue as to the possible presence of this disease. In the examination of a spinal fluid for the detection of neurosyphilis, however, the situation is different. Every case of syphilis is a possible case of neurosyphilis. Stated differently, every case of syphilis gives a clinical \pm reaction for neurosyphilis. Therefore, a serological \pm reaction in such a case adds nothing to the clinical syndrome. A serological \pm reaction means that it is neither positive nor negative and does not help the clinician in making any decision in diagnosis or in treatment of neurosyphilis.

3. This point of view led me to eliminate the \pm reaction from the spinal fluid procedure with the Kahn test. In the "Outline of Technique" of this test (see Annex 2) the \pm reaction was not listed. It is true that I reported a small number of \pm reactions with spinal fluids. The reason for this was that I was working under new conditions and, in addition, I did not know the results of my spinal fluid examinations until the end of the comparison. For these reasons I was moved to practise ultra-conservatism in my reports, and positive reactions which were in any way questionable I reported \pm .

4. In my opinion, two groups of figures taken from Colonel Harrison's tables are significant in this comparison:

Group I. — Variations in B.-W. (Harrison) and Kahn Tests.

In 317 clear (non-contaminated) spinal fluids:

B.-W. positive (++) or (+), Kahn negative (or doubtful)	11
Kahn positive (++) or (+), B.W. negative (or doubtful)	19

In 238 cloudy (contaminated) spinal fluids:

B.-W. positive (++) or (+), Kahn negative (or doubtful)	18
Kahn positive (++) or (+), B.-W. negative (or doubtful)	11

Group II. — "Totals" of ++, +, ± and — Reactions of B.-W. and Kahn Tests.

Figures taken from Table A (clear fluids):

B.-W.				Kahn			
++	+	±	—	++	+	±	—
99	30	46	117	129	8	15	140

Figures taken from Table B (cloudy fluids):

B.-W.				Kahn			
++	+	±	—	++	+	±	—
78	33	27	78	94	10	12	100

Considering that the \pm reactions are of little value and counting them with the negative reactions, the B.-W. test gave a total of 177 ++ and 63 + reactions, while the Kahn test gave a total of 223 ++ and 18 + reactions. Thus the Kahn test gave 46 ++ reactions against 45 + reactions of the B.-W. test.

It is to be noted also from Colonel Harrison's tables that not one of the Kahn \pm reactions were in cases which were frankly non-syphilitic, whereas the B.-W. test gave a few \pm reactions in such cases.

5. There are several technical aspects regarding the Kahn test with spinal fluid which I should like to note here. The test involves precipitating the globulins from the spinal fluid by means of saturated ammonium sulphate and mixing antigen suspension with the dissolved globulin solution. In order to minimise the influence of this salt on the final reaction between the globulin solution and antigen suspension, two steps are employed which tend to lessen the sensitiveness of the reactions: (1) The use of 40 instead of 50 per cent saturation of the spinal fluid with ammonium sulphate, thus reducing the amount of specific globulin in the test; (2) the use of an amount of physiological salt solution in the antigen suspension

greater than that indicated by the antigen titer for tests with serum, thereby reducing the sensitiveness of the antigen suspension.

It was observed that, by employing small test tubes (7.5 by 1 cm.) instead of large ones for mixing the spinal fluid with the saturated ammonium sulphate and centrifuging in the usual manner, it was possible almost completely to remove the supernatant fluid, thus reducing the amount of the salt in the dissolved globulin solution to a minimum. This made possible the use of a 50 instead of 40 per cent. saturation of the spinal fluids with ammonium sulphate. It also made possible the use of an antigen titer similar to that employed in tests with serum. By this method, reactions have been obtained of higher sensitiveness than heretofore without affecting their specificity.

It was also found that sensitised antigen can be employed in the spinal fluid procedure instead of standard antigen — using the same sensitised antigen titer as in tests with serum. The resulting reactions indicate a still higher degree of sensitiveness with freedom from false reactions. Recently, a still more sensitive spinal fluid reaction was obtained with sensitised antigen after increasing the globulin concentration of these fluids to twenty instead of ten times the original concentration of the fluid. This procedure also showed no tendency toward false reactions. It is thus evident that the Kahn reaction makes possible the use of spinal fluid procedures of marked sensitiveness and specificity in the detection of neurosyphilis.

6. I should like to take this opportunity of expressing my obligation and thanks to Colonel Harrison for the cordiality and helpfulness which he kindly extended to me during this comparison. I should also like to thank Dr. J. E. Wyler for helpful co-operation and especially Dr. T. E. Osmond for giving me the freedom of his laboratory and for making my work a most pleasant experience.

REMARKS CONCERNING DR. KAHN'S STATEMENT
BY DR. E. J. WYLER.

Dr. Kahn's 2nd paragraph.

It is stated that, "in the examination of a serum for the detection of syphilis, a \pm reaction is of some value. A clinician may not suspect syphilis and a reaction of this type may give him a clue as to the possible presence of this disease."

Practical experience has shown that \pm reactions, whether with serum or with spinal fluid, are not merely of 'some value', and that their significance, particularly when recurring in subsequent tests, is very considerable, not only for diagnostic purposes, but also in following the effect of treatment. This has been abundantly confirmed in cases where simultaneous tests with flocculation reactions have yielded similar border-line or positive results.

It is also necessary to emphasise that a \pm result is never, in this laboratory, regarded as positive for diagnostic purposes, but as an indication for further clinical and serological investigation. It is interpreted as positive only in known cases of syphilis.

Dr. Kahn's 3rd paragraph.

It is stated that ". . . in addition, I did not know the results of my spinal fluid examinations until the end of the comparison. For these reasons, I was moved to practise ultra-conservatism in my reports, and positive results which were in any way questionable I reported \pm ."

I was of course also unaware of the clinical nature of the specimens until the end of the comparison, and, from the character of the investigation, had also to be conservative in my reports.

Dr. Kahn's 4th paragraph.

Group I appears to illustrate the fact that the Kahn test is under a disadvantage where cloudy fluids are concerned. This is of practical importance.

Under Group II, it is shown that "the Kahn test gave 46 ++ reactions against 45 + reactions of the B.-W. test". It is, however, even less possible thus strictly to compare a ++ complement fixation result with a ++ flocculation test result than to compare a ++ obtained with one B.-W. technique with that obtained by another B.-W. technique, and the statement is therefore not evidence of the greater delicacy of the Kahn test. A strictly accurate comparison of positive results can be obtained only by comparing results called *positive* with one another, irrespective of hieroglyphics. In this laboratory, a result of ++ or + indicates that the serum or spinal fluid is positive for diagnostic purposes.

When the figures of Group II are compared in this way, irrespective of the number of plus signs, it is seen that the Kahn test gave 241 *positive* results against 240 *positive* results by the B.-W. test. But when the ± results, which, in our view, are of great practical importance, are included in the positive results (and as stated above, they are so interpreted in known cases of syphilis), then it is seen that the Kahn test gave 268 *positive* results against 313 *positive* results by the B.-W. test.

With reference to the final sentence of paragraph 4 in which it is stated that the B.-W. test gave "a few" ± reactions in non-syphilitic cases, it is necessary to point out that, out of four such results obtained among the 555 tests, two only were frankly non-syphilitic (= 0.36%), whilst two were clinically doubtful as against one ± result with the Kahn test which was also clinically doubtful. I would here again emphasise that a ± result is never accepted as positive for diagnostic purposes in this laboratory.

Dr. Kahn's 5th paragraph.

Dr. Kahn's investigations with a view to increasing the sensitiveness of the reaction with spinal fluid are of the greatest interest and importance. It must, however, be remarked, in point of laboratory technique, that, while the procedure is far simpler and quicker than the B.-W. test in regard to sera, much of the simplicity and speed is lost where spinal fluids are concerned.

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